COMPARETIVE STUDY OF ANTI DIABETIC AND HYPOLIPIDEMIC ACTIVITY OF CRATEVA MAGNA ROOT AND EUGENIA JAMBOLANA SEED AND ITS COMBINATION IN RATS

A Dissertation submitted to
THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY
CHENNAI 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted By

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Under the guidance of

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CHERRAAN S COLLEGE OF PHARMACY
COIMBATORE-641039
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CHERRAAN S COLLEGE OF PHARMACY

(Affiliated to the Tamilnadu Dr. M.G.R Medical University, Chennai)

Approved by the Govt. of Tamilnadu, Chennai
All India Council for Technical Education, New Delhi
Recognized by Pharmacy Council of India, New Delhi

CERTIFICATE

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This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **COMPARETIVE STUDY OF Antidiabetic and Hypolipidemic activity of Crateva magna root and Eugenia jambolana seed and its combination in rats** submitted by Register no : 261425853 to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy in Pharmacology** is the bonafide work carried out under the guidance and direct supervision of Dr. S. Babuji M.Pharm.,Ph.D., Professor & Head, Department of Pharmacology, and was evaluated by us during the academic year 2016-2017

1.INTERNAL EXAMINER **2. EXTERNAL EXAMINER**

ACKNOWLEDGEMENT

The secret of success is undaunted ardour, motivation, dedication, confidence on self and above all the blessings of god. I bow in reverence to the almighty for bestowing upon me all his kindness that has helped me throughout the journey of my life. Success is an outcome of collaborated efforts aimed that achieving different goals. I hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

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JEYAVEL PANDIAN R

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LIST OF ABBREVIATIONS

ANOVA	:	Analysis of Variance.
ATP	:	Adenosine Triphosphate.
DM	:	Diabetes Mellitus.
GLUT	:	Glucose Transporter.
HDL	:	High Density Lipoprotein.
HbA_{1C}	:	Glycosylated Hemoglobin.
HAECMR	:	Hydro Alcoholic Extract of *Crateva magna root.*
HAEEJS	:	Hydro Alcoholic Extract of *Eugenia jambolana seed.*
HAECOM	:	Hydro Alcoholic Extract of 1:1 Combination.
IDDM	:	Insulin Dependent Diabetes Mellitus.
LD_{50}	:	Median lethal dose.
LDL	:	Low Density lipoprotein.
NOAEL	:	Non Observed Adverse Limit.
NIDDM	:	Non Insulin Dependent Diabetes Mellitus.
OGTT	:	Oral Glucose Tolerance Test.
OECD	:	Organization for Economic Co-operation and Development.
SEM	:	Standard Error Mean.
TG	:	Triglycerides.
TC	:	Total Cholesterol.
VLDL	:	Very Low Density Lipoprotein.
WHO	:	World Health Organisation.
dL	:	decilitre.
Kg	:	kilogram.
mg	:	milligram.
ml	:	millilitre.
p.o.	:	per oral.

1. INTRODUCTION

The metabolic disease diabetes mellitus and its incidents keeps on increasing in our nation at a fastest rate. So, that as many as 50 million people are at present suffering from type II diabetes and We the health providers have to face this challenge. Exactly for this particular reason, I have choosen this project work entitled Comparetive study of Antidiabetic & Hypolipidemic activity of Crateva magna root & Eugenia jambolana seed and its combination in rats . Diabetes is one of the most talked about diseases across the world and especially in India, yet the awareness about the same do not exist among the people sufficiently. With the country having the highest number of diabetic patients in the world, the sugar disease is posing an enormous health problem today in our nation. India , often known as the diabetes capital of the world, has been witnessing an alarming rise in the incidence of diabetes according to the International Journal of Diabetes in Developing Countries. According to a World Health Organization (WHO) fact sheet on diabetes, an estimated 3.4 million deaths are caused due to diabetes.

The WHO also estimates that 80 per cent of diabetes deaths occur in low and middle-income countries and projects that such deaths will double between 2016 and 2030. It has been further estimated that the global burden of type-2 diabetes is expected to increase to 438 million by 2030 from 285 million people (recorded in 2010). Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030. But debates, discussions and deliberations aside, the fundamental thing is to know what exactly is diabetes.

Diabetes mellitus is a group of chronic diseases characterized by high levels of blood glucose caused by body s inability to produce or the body s cells unresponsiveness to insulin. Diabetes can lead to blindness, kidney failure, nerve damage and blood vessels. It plays an important factor in accelerating the hardening and narrowing of arteries (atherosclerosis), leading to strokes, coronary heart disease.

Diabetes is one of the first diseases described with an Egyptian manuscript from 1500BC, mentioning too great emptying of urine . The described cases are believed to be of type 1 Diabetes. Indian physicians around the same time identified the disease and classified it as *madhumeha* or *honey urine* noting that the urine would attract ants. The term Diabetes or to pass through was first used in 230 BC by the Greek Appollonius of Memphis. The disease was rare during the time of the Roman empire with Galen commenting that he had only two cases

during the time of the Roman empire with Galen commenting that he had only two cases during the career. Type 1 and type 2 Diabetes where identified as separate conditions for the first time by the physicians Sushrutha and Charaka in 400-500 AD with type 1 associated with youth and type 2 with overweight. The term Mellitus or from honey was added by the Britain John Rolle in the late 1700s to separate the condition from Diabetes insipidus which is also associated frequent urination. Until early part of 20th century there was no effective treatment for Diabetes. Canadians Frederick Banting and Charles Best developed insulin in 1921 and 1922. This was followed by developed of long lasting insulin NPH in 1940s. [1,2]

According to World Health Organisation[79]

- 346 million people in world are suffering with Diabetes.
- In 2004, it is estimated that 3.4 million people died from consequences of high blood sugar.
- More than 80% of Diabetes deaths occur in low and middle income countries.
- WHO projects that Diabetes deaths will double between 2005 and 2030.
- Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type 2 diabetes.

1.1 HERBAL DRUGS IN DIABETES TREATMENT

India is the largest producer of medicinal herbs and is called as botanical garden of the world. Ayurveda, Unani, Siddha and Folk (also called tribal) medicines are the major systems of Indian medicines. Among these systems, Ayurveda is most developed and widely practiced in India. A number of plants have been described in Ayurveda and other traditional medicine for the treatment of diabetes. Isolation of the main compounds from the active extract is a crucial step in all research activities for developing a novel phytomedicine for diabetes mellitus.

A number of plants have been described in Ayurveda and other traditional medicine for the treatment of Diabetes. In Ayurveda Diabetes is described as Madhumeha . In developing countries 80 % of populations are using traditional medicine in primary medical problems. Drug Metformin (a biguanide) is a derivative of an active natural product, galagine a biguanide isolated from the plant Galega officinalis L., which was used to relieve the intense urination in diabetic people. Even the root juice of Helicteris isora Linn (Family-Sterculaceae) is claimed to be useful in Diabetes, emphysema and a favourite cure for snake bite. Despite considerable progress in the management of Diabetes by conventional drugs, the search for natural antidiabetic plant products

as alternative therapy for progressing . Many medicinal plants have been shown experimental or clinical antidiabetic activity and that have been used in traditional systems of medicine. Plants such as M. Charantia, Eugenia Jambolona, Mucuna precriens, Murraya Koeingii and Brassica have found to have anti diabetic activity. All plants have shown varying degree of hypolipidemic and antihyperglycemic activity. In the recent years more than 500 herbal medicines have been reported to possess anti-diabetic property.[3-8]

An ethno botanical survey of semi-structured questionnaire of medicinal plants in five districts of Nigeria reputed for the treatment of Diabetes have been reported. The principal antidiabetic plants included Vernonia amygdalina and Morinda lucida. The data base maintaining the record of medicinal plants having hypoglycemia or antidiabetic has been reported on 2009. The herbal drugs with antidiabetic activity are yet to be commercially formulated as poly herbal formulation, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine.

The management of diabetes without any side effect is still a challenge to the medical system. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. Wide array of plant derived active principles have demonstrated anti diabetic activity. The main active constituents of these plants include alkaloids, glycosides, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. These affect various metabolic cascades, which directly or indirectly affect the level of glucose in the humanbody.[9]

World Health Organisation recommendations (WHO 1980) on the use of alternative medicines for treating diabetes mellitus provide an impetus for research in this area. Currently, the focus of research in diabetes includes discovering newer anti diabetic agents as well as isolating the active compounds from herbal sources that have been documented to have anti diabetic properties as have been described in ancient texts.[10]

1.1. The scientifically documented list of medicinal plants exhibiting antidiabetic activity.

Table no: 1. List of medicinal plants exhibiting antidiabetic activity

Name of the plant	Family	Parts used	Type of the plant extract	Animal model	Reference
Aloe vera	Asphodelaceae	Leaf, gel extract	Ethanolic	Rats (alx)	26
Artemisia sphaerocephala krasch	Astaraceae	Seeds	Aqueous	Rats (alx)	27
Artanema sesamoides	Scophuilariaceae	Aerial parts	Methanolic	Rats (stz)	28
Cassia kleinii	Caesalpiniaceae	Leaf, roots	Aqueous, Ethanolic	Rats (alx)	29
Cassia glauca Linn.	Caesalpiniaceae	Leaves	Petroleumether, chloroform, acetone, methanolic	Rats (stz)	30
Coctus pictus	Zingiberaceae	Leaves	Aqueous	Rats (stz)	31
Ficus hispida	Moraceae	Bark	Ethanolic	Rats (alx)	32
Lycium barbarum	Solanaceae	Fruits	Aqueous	Rats (alx)	33
Eugenia jambolona	Myrtaceae	Fruits	Aqueous, ethanolic	Rats (alx)	34
Ficus	Moraceae	Leaves	Ethanolic	Rats (alx)	35

microcarpa					
Phyllanthus rheedii	Euphorbiaceae	Whole plant	Ethanolic	Rats (stz)	36
Justicia beddomei	Acanthaceaea	Leaves	Chloroform, ethanolic	Rats (alx)	37
Nymphaea stellata	Nymphaeaceae	Flowers	Hydro-ethanolic	Rats (alx)	38
Ichonocarpus fructescens	Apocynaceae	Roots	Aqueous	Rats (stz)	39
Salvadora oleoides	Salvadora oleoides	Stems, leaves	Ethanolic	Rats (alx)	40
Adhatoda zeylanica Medic	Acanthacaeae	Leaves	Hexane, Methanolic	Rats (alx)	41
Berberis aristata D.C	Berberidaceae	Roots	Ethanolic, chloroform, petroleum ether	Rats (alx)	42
Gymnema sylvestre	Asclepiadaceae	Leaves	Aqueous	Rats (alx)	43
Tinospora cordifolia Willd	Menipermacadeae	Stem	Aqueous and ethanolic	Rats (stz)	44
Neolamarckia cadamba	Rubiaceae	Stem, Bark	Ethanolic	Rats (alx)	45

Barleria prionitis	Acanthaceae	Leaves, roots	Ethanolic	Rats (alx)	46
Pongamia pinnata	Leguminosae	Leaves	Ethanolic, Chloroform	Rats (alx)	47
Vinca rosea	Apocynaceae	Leaves	Dichloro-methane and methanolic	Rats (alx)	48
Vinca rosea	Apocynaceae	Whole plant	Ethanolic	Rats (alx)	49
Indigofera pulchra	Papilionaceae	Leaves	Ethyl acetate, n-butanol	Rats (alx)	50
Sarcococca saligna	Buxaceae	Whole plant	Petroleum ether, ethyl acetate	Rats (stz)	51
Momordica charantia	Cucurbitaceae	Fruits, leaves	Ethanolic	Rats (alx)	52
Holostemma ada-kodien	Asclepiadaceae	Root	Ethanolic	Rats (alx)	53

2. SCOPE OF THE PRESENT STUDY

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. The hyperglycemia results from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of Diabetes is associated with specific chronic complications resulting in damage to or failure of various organs, notably the eyes, kidneys, heart and blood vessels.

Diabetes mellitus is set to become one of the worlds biggest health problems owing to the projected increase in new cases. In India, the prevalence rate of Diabetes is estimated to be 1-5%. Complications are the major cause of morbidity and mortality in Diabetes mellitus.

So many herbal drugs are used in the treatment of Diabetes mellitus. *Crateva magna* is having the anti diabetic activity. As per the literature review still no anti diabetic activity has been reported on this plant. In review of literature *Crateva nurvella* has shown antidiabetic activity. So, the *Crateva magna* also has come from the same family. Hence, this study has been taken to explore the antidiabetic, hypolipidemic potential of *Crateva magna root* on streptozotocin induced Diabetes in Wistar albino rats and Eugenia jambolana seed has well known for its anti diabetic activity.

For the present study I have selected 3 samples of herbal drugs for screening of antidiabetic activity,

1. *Crateva magna* root.
2. *Eugenia jambolana* seed.
3. 1:1 Combination of *Crateva magna* root and *Eugenia jambolana* seed.

2.1 PLANT DESCRIPTION

1. *Crateva magna*

Botanical Name : *Crateva magna*

Family : Capparaceae

Trade Name :Three Leaved Caper, Garlic PearTree

Vernacular Names

English : Three Leaved Caper, Garlic PearTree

Tamil Name : Mavilangam, Varanam

Malayalam : Neermathalam, Neerval, Mavulangam

Hindi Name : Barun, Barna

Sanskrit Name : Varuna

Taxonomical Classification

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Brassicales

Family : Cappraceae

Genus : Crateva

Species : *Crateva magna*

Scientific Name : *Crateva magna*

Picture No : 1 Flowers of *Crateva magna*

Picture No : 2 Whole Plant of *Crateva Magna*

Botanical Description :

A small much branched deciduous tree. Leaves 3-foliolate; leaflets 5-15 cm long, ovate, lanceolate or obovate, acute. Flowers many, in dense terminal corymbs; petals nearly 2.5 cm long, greenish white; stamens longer than the petals. Fruit a globose or ovoid, woody berry. Trees, to 10 m high, bark 2-3 mm thick, surface grey, smooth, longitudinally wrinkled; blaze dull yellow. Leaves digitately trifoliate, alternate, estipulate; rachis 4-12 cm long, slender, grooved above, glabrous; petiolule 4-7 mm long, slender; leaflets 10-20 x 3.5-6 cm, unequal, ovate, ovate-lanceolate or broadly elliptic, base acute, or subacute, apex acute or acuminate, margin entire, glabrous, coriaceous; lateral nerves 9-15 pairs, slender, parallel, prominent, intercostae reticulate, faint. Flowers bisexual, creamy white, in terminal corymbs; pedicel to 5 cm long; sepals 4, 3 mm long, free, oblong, adnate to the lobed disc; petals 4, ca. 2 cm long, creamy white, often tinged with purple, long-clawed, obovate; disc incurved, nectariferous; stamens many, free, longer than the petals, inserted at the base of gynophore; anthers basifixed, filaments spreading; gynophore 4-5 cm long at anthesis, slender; ovary superior, ellipsoid, 1-[2]-celled, ovules many; stigma sessile, capitate. Fruit a berry, globose or ovoid, yellowish-grey, rind woody, scurfy, 4-5 x 2.5-3.5 cm; seeds brown, embedded in pulp.

Biology

Crateva magna is small wild or cultivated tree native to india, China, Bangaladesh, Sri Lanka, Burma, Thailand, Campodia, Indonesia and Malaysia. Its often found along streams also in dry, deep boulder formations in Sub Himalayan tracts.

Habit : Tree

Flowering & Fruiting : March April

Habitat : River banks semi evergreen forests

Distribution : Indo-malaysian, China

Aquatic : No

Epiphyte(s) : No

Flower Colour : Creamy White

Weed : No

Monocot/Dicot : Dicotyledonous Plants

Chemical Constituents

Stem bark of the plant contains saponins, flavonoids, sterols and glucosilinates and ceryl alcohol, friedelin, cadabicine, diacetate, lupeol, betulinic acid and diosgenin. Fruits contain glycocaparin, ß-sitosterol, triacontane, triacontanol, cetyl and ceryl alcohols. Leaves contain l-stachydrine. Root bark contains rutin, quercetin, lupeol, varunol and ß-sitosterol.

It also contains lauric, stearic, undecylic, oleic and linolenic acids and a new triterpene alcohol lupa-21, 20(29)dien-3ß-ol. Presence of alkaloids have been reported in bark and stem. These alkaloids showed antimicrobial activities. Fruit contains cetyl alcohol, ceryl alcohol, triacontane, triacontanol, ß-sitosterol and glucocapparin (Ghani, 2003).

Medicinal uses

The dried bark is used raw drug in traditional systems of medicine in India such as Ayurveda, siddha etc. The decoction of bark is internally administered to cure diseases like renal calculi, dysuria, helminthiasis, inflammations and abscesses. The decoction exhibits actions like carminative, laxative, thermogenic, diuretic, lithontriptic, expectorant and demulcent.[4] The leaf and stem bark have been evaluated for their antioxidant activity and inhibition of key enzymes relevant to hyperglycemia.[5] Leaves are stomachic, tonic, rubefacient and febrifuge; used in rheumatism. Flowers are astringent and cholagogue. A couple of buds pounded with salt are taken before meals to promote the appetite. The fruits are laxative. Roots and bark are laxative, lithontriptic, anthelmintic, expectorant; promote appetite and increases biliary secretion.

Bark is demulcent, stomachic and laxative; useful in fever, vomiting and symptoms of gastric irritation; especially useful in cases of kidney and bladder stones. Juice of the bark is given to women as a contraceptive after childbirth. Bark is bitter and poisonous. Water soluble fraction of alcoholic extract is spasmolytic on animal and human uteri and cholinergic on smooth muscles of animals. Petrolium ether extract is anti-inflammatory against different types of inflammation. Root extract is antibacterial against Gram +ve and Gram ve bacteria. Alcoholic extract of stem is antibacterial against E. coli. Plant inhibited phosphatic stone formation in rats (Asolkar et al., 1992).

2. Eugenia jambolana

Botanical Name : *Eugenia jambolana*

Family : myrtaceae

Trade Name : jaman

Vernacular Names

English : jaman

Tamil Name : naval

Gujarati : jambu

Hindi Name : jamun

Sanskrit Name : jambu

Kannada Name : Ama-Phala, Jambunerale, Nayenerale

Marathi Name : Jambhul

Telugu Name : Goyya Pandu, Jam-Pandu

Taxonomical Classification

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

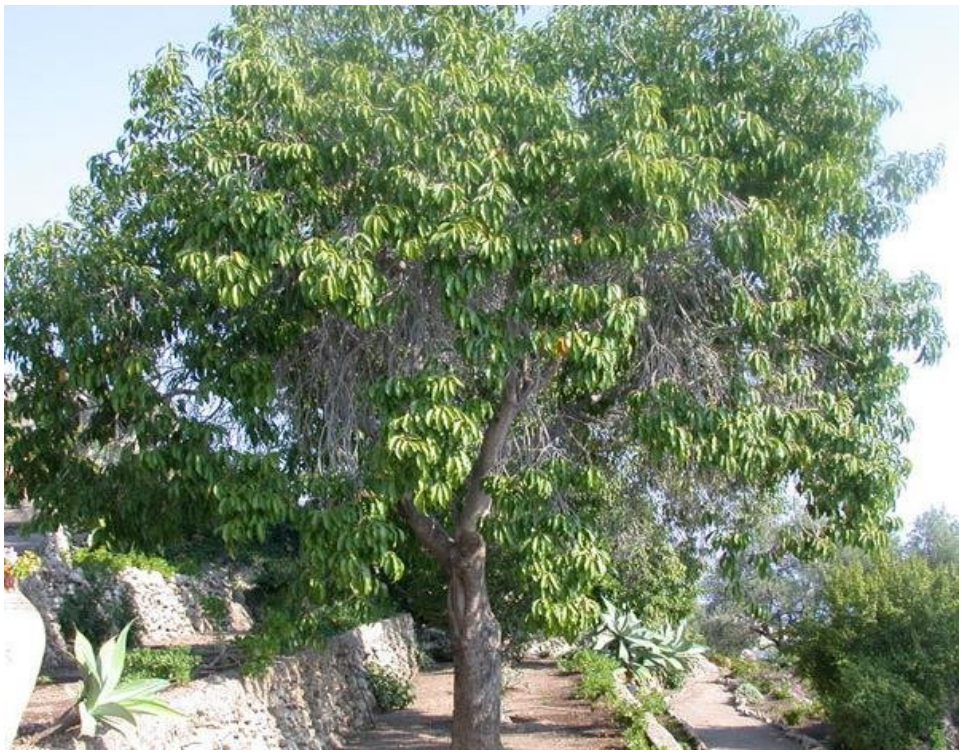
Order : Myrtales

Family : Myrtaceae

Genus : Eugenia

Species : jambolana

Scientific Name : *Eugenia jambolana*



Picture No 3: *Eugenia jambolana* Whole Tree

Picture No 4: Fruits of *Eugenia jambolana*

Biology

It is an ever green tree that attains a height of 100 feet and can be 12 feet broad. Leaves 3 to 6 inch long and 2 to 3 inch broad. These are ovate shape and are shiny in texture. Flowers are light green to white in colour. Fruits are ½ to 1½ inch long and oval in shape. When the fruit is raw it is green in colour when it is ripe it becomes red or purple in colour. The fruit contains seed that is about 1 to 2 cm in length. Flowers are born in early summers and fruits in autumn.

Chemical Constituents

Fruits has contains moisture that is 83.7%, protein 0.7%, fat 0.3%, carbohydrate 14%, minerals, vitamin A,B,C, melic acid, oxalic acid, garlic acid, tannin, a aromatic oil and a blue colour substance which is cyaniding diglycosides. Besides it contains tannins 19%, elgic acid, gallic acid 1 to 2% and glycoside named jamboline. It also contains starchand light yellow coloured aromatic oil which is 0.05%. Bark contains betulenic acid, beta sitosterol, elagic acid and mirisetin.

Pharmacological Use

It is useful in ailments caused by kapha and pitta. It has a cohesive action. Because of cool nature it is helpful in skin related ailments and suppresses burning sensation occurring in the body. It helps proper digestion in the body. It helps in curbing infection in the body. It is an antidiuretic in nature and reduces the sugar levels in blood and urine.

3. Review of Literature

3.1. *Crateva magna*

1. Sovan Pattanaik* and et al, School of Pharmaceutical Sciences, Siksha O Anusandhan University, Bhubaneswar, Odisha, India. **Wound healing activity** of methanolic extract of the leaves of *Crateva magna* and Euphorbia nerifolia in rats Abstract *Crataeva magna* and Euphorbia nerifolia have been shown to possess hepatoprotective activity and antioxidant property. The present works with these plants were undertaken with the premise that the drug promoting hepatoprotective activity and radical scavenging property could have effect on wound healing also. The wound healing property of the methanolic extract of the leaves of *Crataeva magna* (CNM) and Euphorbia nerifolia (ENM) were chosen to investigate in excision and incision wound models. The methanolic extracts of the two plants at the dose of 500 mg/kg/day by topically applying method. Healing was assessed by the rate of wound contraction, time until complete epithelialization, incision breaking strength, estimation of hydroxyproline and histopathological parameters. Complete wound contraction was shown by both the plants in the study period. In excision and incision wound models, all the test drugs showed significant ($P < 0.001$) wound healing activities compared to the control. Moreover the CNM was found to possess significant wound healing activities than the ENM and had been observed to have equipotent wound healing activity as of the standard drug Framycetin.

2. Atanu Bhattacharjee and et al, Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore, Karnataka, India **Phytochemical and ethno-pharmacological profile** of *Crataeva nurvala* Buch-Hum (Varuna).

Crataeva nurvala Buch-Hum (Varuna) is well known traditional Indian medicinal to treat various ailments in particular urolithiasis. During last two decades, numerous ethno-pharmacological and scientific reports have been cited in the literature to support its multidirectional therapeutic potential. The plant is rich in alkaloids, Saponins, triterpenes, tannins, flavanoid glycosides, glycosinolates and phytosterols. The review emphasizes primarily on folkloric uses, biologic activities of isolated compounds, pharmacological activities of the extracts, clinical studies and safety profile of crateva nurvella to provide a comprehensive data for researchers to hit upon new chemical entity responsible for its claimed traditional uses.

3. Shashikanta behera and et al Department of Botany, Ravenshaw University, Cuttack, Odisha, India. **Phytochemical analysis of the leaf, flower, and bark extracts** was done using various solvent by standard methods as described by Harborne (1973). Six different extracts each

for leaf, flower, and bark were screened. Saponins were present in all the extracts of leaf, flower, and bark, whereas not a single extract showed the presence of steroids. Five extracts of flower and four extracts of leaf and bark indicated the prevalence of flavonoids. Similarly, four extracts of bark and three extracts of leaf and flower showed the incidence of tannins. Terpenoids occurred only in the aqueous extracts of leaf and flower, whereas alkaloids were found only in two and one extract(s) of leaf and bark, respectively. Phenolic compounds were not found in any of the bark extracts, but three and two extracts of leaf and flower showed their presence.

4. Sridhar N and et al, **Antinephrolithiatic effect of *Crateva magna* Lour DC** root on ethylene glycol induced lithiasis. The extract of *Crateva magna* was screened for antinephrolithiatic activity in male wister rats were summarised based on the ionic changes in both urine and serum. The present study was carried out to evaluate Antinephrolitiatic activity in rats using 0.75% ethylene glycol in drinking water was given orally for 28 days. The plant *Crateva magna* is used in analgesic, antiprotozoal, hypoglycaemic, anti-inflammatory, hypotensive, anti spasmodic purposes. The aqueous extract of leaves of *Crateva magna* results maximum yield value than that of petroleum ether extract, Chloroform extract and alcohol extract through successive maceration process. The aqueous extract of leaves of *Crateva magna* showed maximum control of lithiasis in wister rats. Urinary volume was increased in nephrolithiatic as well as drug treated rats. Increased urinary excretion of calcium, oxalate, uric acid, phosphates and protein in nephrolithiatic rats was bought down significantly by the administration of *Crateva magna*. Decreased magnesium excretion in hyperoxaluric rats was normalized with respect to calcium oxalate and other crystallizing salts such as uric acid, which may induce epitaxial deposition of calcium oxalate. Simultaneous treatment with the extract reduced calcium and oxalate ion concentration in urine confirming stone inhibitory effect. In Ethylene glycol induced nephrolithiasis, the nephrolithisis was significantly reduced and the stone formation was normalized by administration 200 mg/kg and 400 mg/kg dose orally and the property was comparable to the standard drug. This study has extablished the antinephrolithiatic activity of *Crateva magna* and thus, justifies the uses of this plan lithiasis.

5. Amresh Panda and et al, **Hepatoprotective activity of leaves of *Crataeva magna*** in different types of hepatotoxic rat models. *Crataeva magna* (Lour.) DC belonging to family Capparaceae is a high-value medium sized deciduous medicinal tree of tropical climate found in tropical regions of the world and also grows almost all over India, especially in the semiarid regions. The present

investigation is designed to conduct phytochemical screening of *Crataeva magna* leaves after each successive extraction with petroleum ether, chloroform, methanol and water followed by its hepatoprotective activity study. Three different types of models used to examine the in vivo hepatoprotective activity of the above said extract were carbon tetrachloride, ethanol and paracetamol induced hepatotoxicity in rats and compared with silymarin (20 mg/kg) as reference standard. Phytochemical screening revealed the presence of carbohydrates, phenolic compounds, tannins, flavonoids, saponins and fixed oils in the aqueous extract of the *Crataeva magna* leaves. Two way analysis of variance study of the estimated biochemical parameters to illustrate, aspartate aminotransferase, alanine amino transferase and alkaline phosphatase were revealed that there is significant difference (p-value < 0.001) exists between the different treatment groups. Severe hepatic lesions induced by carbon tetrachloride, ethanol and paracetamol were significantly lowered after the administration of CM 200 mg/kg to the respective control groups (carbon tetrachloride > paracetamol > ethanol) which was also evident from the histopathological study of liver sections.

6. Sridhar N and et al, ***In-vitro antioxidant activity of Crataeva magna lour. Dc bark extract***. The plant *Crataeva magna* belonging to family Capparaceae is used in anti spasmodic, hypotensive, anti-inflammatory, hypoglycemic, anti protozoal, analgesic purposes. The present study was carried out to evaluate appropriate animal model. The antioxidative potential of different solvent extracts of *Crataeva magna* were evaluated using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), 2,2′-Azino-Bis(3-ethylbenzthiazoline-6-Sulphonic acid) (ABTS), superoxide radical, hydroxyl radical, nitric oxide radical scavenging activities and lipid peroxidation inhibition assay. Among those solvent extracts, ethanolic extract of *C. magna* exhibited highest level of antioxidant activities. The ethanolic extract also inhibited H2O2 mediated haemolysis and lipid peroxidation in human RBC.

7. S. Pattanaik and et al, ***Evaluation of hepatoprotectve activity*** *and isolation of 2-(3, 4-Dihydroxy Phenyl)-7-Hydroxy-3-(2-Hydroxy Ethoxy) 4-H-chromen-4one from column fractions of leaves of the extract of Crataeva magna.* Nature is only source of hepatoprotective drugs in modern medicine to prevent and treat drug-induced liver damage. The alcoholic extract of fresh leaves of the plant Crataeva magna, previously reported for its hepatoprotective activity was fractionated into three parts to chemically identify the most potent bioactive fraction. The current study was designed to evaluate the hepatoprotective activity, isolation and characterization property of effective column fractions of the leaves of the plant *Crataeva magna*. The hepatopreventive effect of the column fractions (F1, F2 and F3) of the leaves of the plant were evaluated by measuring the levels of serum liver damage marker enzyme

such as alanine transaminase, aspartate transaminase, alkaline phosphatase, total and direct bilurubin. As per the result, since the F3 fraction of the extract showing out the significant reduction in the elevated serum level which can be compared with the standard drug silymarin therefore, phytochemical investigation of the F3 fractions led to the identify the structure of the flavonoid compound which was established by spectroscopic methods (UV, IR, 1H NMR, 13C NMR and EI-MS) as 2-(3, 4-Dihydroxy Phenyl)-7-Hydroxy-3-(2-Hydroxy Ethoxy) 4-H-Chromen-4one.

8. Chidambaram K and et al, **Antipyretic activity of *Crateva magna*** bark on Tab-Vaccine induced pyrexia Objective: *Crataeva magna* is a potent medicinal plant in the Indian systems of medicine. Traditionally used for inflammation, fever, arthritis, bronchitis, urinary calculi and cough. The objective of the present work was to study the antipyretic activity of plant *Crataeva magna* (Lour.) DC belonging to family Capparaceae. Materials & Methods: In the present study the alcoholic extracts of the bark of *Crataeva magna* were studied for their antipyretic activity by TAB (Typhoid) vaccine-induced pyrexia in rabbits. Result: In TAB vaccine-induced fever, the fever was significantly reduced and the body temperature was normalized by administration of 200 and 400 mg/kg dose orally and the property was comparable to the reference drug. Conclusion: This study has established the antipyretic activity of *Crataeva magna* and thus, justifies the anecdotal, folkloric and ethnomedical uses of this plant for fever.

9. Monnanda S Nalini and et al, **Fungal endophytes** from the three-leaved caper, *Crataeva magna* (Lour.) DC. (Capparidaceae) Fungal endophytes were isolated from Crataeva magna, a medicinal plant growing along the streams and rivers, constituting riparian vegetation in Karnataka, southern India. Fresh bark and twig pieces were used for the isolation using standard methods. Ninety-six endophytic fungal isolates were isolated from 800 bark and twig segments. Mitosporic fungi represented as a major group (85%) followed by zygomycetes (10%) and ascomycetes (5%). Bark samples contained more endophytes than twig samples. Verticillium, Nigrospora oryzae and Fusarium verticilloides were the dominant fungal endophytes.

10. Nishritha Bopana and et al, **In vitro regeneration of clonally uniform plants of *Crataeva magna***: A high value medicinal tree by axillary branching method. *Crataeva magna* (Lour.) DC (synonym C. nurvala) is a high-value Indian medicinal tree. The multiple uses of *C. magna* have resulted in its over-exploitation. Erratic seed germination combined with destructive harvesting and habitat destruction in the form of deforestation has added to the enormity of the problem. Therefore, the need for conservation of this plant is vital. Development of an efficient

micropropagation protocol will play a significant role in meeting the requirement of quality planting material for commercial cultivation thereby conserving the species in its natural habitat. In the present study, shoot multiplication was achieved by culturing single node segments derived from a field grown tree on Murashige and Skoog s (MS) medium supplemented with 2.66µM N6-benzyladenine, 1.39µM Kinetin (Kn), 0.57µM indole-3-acetic acid (IAA), 3% sucrose and 0.2% gelrite. 96% rooting was achieved within 22days by culturing the in vitro formed shoots on half strength MS medium with 11.42µM IAA, 9.8µM indole-3-butyric acid, 0.46µM Kn and 198.25µM phloroglucinol. Following a simple hardening procedure involving sequential transfer of plants to a greenhouse, polyhouse, and shade net, the tissue-cultured plants were transferred to the field where the survival rate was 100%. To this date 500 plants have been produced. Inter simple sequence repeat analysis has confirmed genetic uniformity of the tissue-cultured plants.

3.2. Eugenia jambolana

1. Ravi Kasiappan and et al, Anti-Diabetic Activity of *Eugenia jambolana* Seed Kernels on Streptozotocin-Induced Diabetic Rats .The present study evaluated the hypoglycemic activity of different parts of *Eugenia jambolana* seeds such as whole seed, kernel, and seed coat on streptozotocin-induced diabetic rats. Administration of the ethanolic extract of kernel at a concentration of 100 mg/kg of body weight significantly decreased the levels of blood glucose, blood urea, and cholesterol, increased glucose tolerance and levels of total proteins and liver glycogen, and decreased the activities of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase in experimental diabetic rats. Whole seed showed a moderate hypoglycemic effect, and seed coat did not show any hypoglycemic effect. The hypoglycemic efficacy was compared with that of glibenclamide, a standard hypoglycemic drug.
2. M.T. Pepato and et al, Lack of antidiabetic effect of a *Eugenia jambolana* leaf decoction on rat streptozotocin diabetes Streptozotocin-diabetic rats were treated for 17 days with a decoction of *Eugenia jambolana* (Myrtaceae) leaves (15%, w/v) as a substitute for water. Body weight, food and fluid intake, urine volume, glycemia, urinary glucose and urea were evaluated every 5 days. The animals were sacrificed by decapitation and blood samples collected for the determination of glycemia, serum cholesterol, HDL-cholesterol, triglycerides and angiotensin-converting enzyme. The weight of adipose and muscle tissues was also determined. There were no statistically significant differences between treated and untreated rats for any of the biochemical or physiological parameters. We conclude that, at least in this experimental model, *Eugenia jambolana* leaf decoction has no antidiabetic activity.

3. Das S and et al, The hepatoprotective activity of the ethanolic extract of the pulp of *Eugenia jambolana* in albino rats.

4. Venkata Charepalli and et al, *Eugenia jambolana* (Java Plum) Fruit extract exhibits anti-Cancer activity against early stage human HCT-116 Colon cancer cells and colon cancer stem cells.

5. Debjith Bowmik and et al, Jamun or Indian Black berry is considered as a traditional medicine that helps in controlling diabetes. Specifically, jamun has an action on the pancreas, the main organ responsible for causing diabetes. The fruit, the seeds and even the juice of the jamun all play an important role in the treatment of diabetes. The jamun seeds contain a type of glucose called Jamboline, which checks the conversion of starch into sugar in cases of increased

production of glucose, the main reason behind your high sugar levels. It has anti-cancer and anti-viral properties. Jamun juice has carminative and mild astringent properties. The extracts of the bark, seeds and leaves are used for the treatment of diabetes. The leaves have antibacterial properties and used for strengthening teeth and gums. Oral administration of dried alcoholic extracts of the seeds to diabetic patients was found to reduce the level of blood sugar and glycosuria in trials conducted at CDRI, Lucknow, India. The bark of black berry tree is astringent, digestive, diuretic, anthelmintic and is considered useful for throat problems. A decoction of the bark and powdered seeds is believed to be very useful in the treatment of diarrhea, dysentery and dyspepsia. The antibiotic activity of black berry extract has been widely studied and found useful against a number of microbial agents. The fruit is also considered to be stomachic, carminative, antiscorbutic and diuretic. Vinegar made from black berry fruit is administered in cases of enlargement of spleen, chronic diarrhoea and urine retention for ringworm treatment, water diluted juice is used as lotion.

6. Sadaf Rasheed and et al, Histological effect of *Eugenia jambolana* seed extract on liver of Adult albino rats.The therapeutic value of *Eugenia jambolana*, commonly known as Jamun in Hindi, has been recognized in different system of traditional medicine for the treatment of various conditions. Its seeds are used for the treatment of diabetes mellitus and hyperlipedemia by reducing the lipid levels in the body; this action is presumed to be due to blocking the action of enzyme 3-hydroxyl methyl glutaryl (HMG-CoA reductase in the liver. Herbal drugs are getting into use with the notion that these are relatively harmless; the practice has shown that many of them also have toxic effects. Since hardly any work is available on the toxic aspect of Eugenia Jamblana, the present study was planed to see the effect of ethanolic extract of Eugenia Jamblana on liver using albino rats as an experimental model.

7. Suman Bala Sharma and et al, Ameliorative effect of active principle isolated from seeds of *Eugenia jambolana* on carbohydrate metabolism in experimental diabetes. The aim of this study was to evaluate the antidiabetic activity of LH II purified from ethanolic seed extract of *Eugenia jambolana* in alloxan-induced mild diabetic (MD) and severely diabetic (SD) rabbits. Ethanolic extract upon chromatographic purification yielded partially purified hypoglycemic principle (SIII) which on further purification by sephadex LH 20 yielded pharmacological active compound LH II. Homogeneity of LH II was tested by HPLC. Phytochemical investigation of LH II by various structural spectra showed the presence of saturated fatty acid, Δ5 lipid and presence of sterol. LH II was administered orally at a dose of 10 mg kg−1 body weight to MD

and SD. LH II resulted, significant fall in FBG at 90 min (21.2% MD: 28.6% SD), 7th day (35.6% MD) and 15th day (59.6% SD). Glycosylated hemoglobin was significantly decreased (50.5%) in SD after 15 days treatment (Tt). Plasma insulin levels were significantly increased ($P < .001$). In vitro studies with pancreatic islets showed 3-fold increase in insulin levels as compared to untreated animals. LH II also showed extrapancreatic effect by significantly increasing ($P < .001$) the activity of key enzymes of glycolysis and significantly decreasing ($P < .001$) the activity of key enzymes of gluconeogenesis. Liver and muscle glycogen content were increased by 36.6 and 30% for MD, and 52 and 47% for SD, respectively. Thus, the present study demonstrates that LH II possesses potent antidiabetic activity and it is effective in both MD and SD rabbits.

3.3. PRECLINICAL IN VIVO ANIMAL MODELS OF DIABETES MELLITUS FOR SCREENING OF POTENTIAL ANTI-DIABETIC ACTIVITY

1. Pharmacological induction of Diabetes Mellitus by using Streptozotocin in rats:

Generally in the laboratories to create diabetogenic effect they will administered streptozotocin (STZ) with regard to their health condition and mortality rates. Normally Eight-week-old rats, weighing from 200 to 230 g, were randomised into five groups of eight animals. Streptozotocin was administered by i.v. injection in doses of 40, 50, 60 and 70 mg/kg body weight. The animals were kept on a standard diet with free access to water for 4 months. The highest STZ dose (70 mg/kg) was lethal to the animals, the doses of 50 and 60 mg/kg induced persistent hyperglycaemia with glucose levels above 20 mM. Body weights of STZ treated rats from all experimental groups were significantly lower than those of control animals. Considerable polyuria was observed in all STZ treated rats. About 40 % of the STZ treated animals were found to develop overt cataract between days 90 and 100. At the end of the experiment, significant albuminuria was observed in the experimental groups administered 50 and 60 mg/kg STZ doses. We conclude that young male rats, Breeding Facility, treated by a single i.v. STZ dose of 50 or 60 mg/kg developed a persistent disease state characterised by severe hyperglycaemia with major clinical signs of diabetes mellitus.

4. AIM AND OBJECTIVES

The present investigation was aimed to screen the anti diabetic and hypolipidemic activity of *Crateva magna* root and *Eugenia jambolana* seed and its combination on diabetic rats.

Objective

The objective of the present study was to,

a. Find out the phytochemical constituents present in the hydroalcoholic extract of the root of *Crateva magna* plant.
b. Find out LD_{50} dose of the hydroalcoholic extract of root of *Crateva magna* plant.
c. Evaluate the anti diabetic, hypolipidemic activity of *Crateva magna* plant.
d. Evaluate the anti diabetic, hypolipidemic activity of *Eugenia jambolana* plant.
e. And evaluate the anti diabetic activity of 1:1 combination of *Crateva magna* root and *Eugenia jambolana* seed.

5. PLAN OF WORK

1. Collection of the plants (*Crateva magna* root and *Eugenia jambolana* seed).
2. Authentification of the plant, shade drying granulation of the root and seed of the plant.
3. Extraction with solvents.
4. Preliminary Phytochemical screening of HAECMR.
5. Carrying out acute toxicity to determine LD_{50} VALUE.
6. Carrying out oral glucose tolerance test.
7. Carrying out the anti-diabetic activity for *Crateva magna* root *Eugenia jambolana* seed.
8. Carrying out hypolipidemic activity for *Crateva magna* root *Eugenia jambolana* seed.
9. Estimation of biochemical parameters for *Crateva magna* root.
10. Determination of atherogenecity.
11. Estimation of body weight.

6. MATERIALS AND METHODS

6.1. PLANT MATERIAL

Root of *Crateva magna* and *Eugenia jambolana* seed was collected from Nallamalla forest, Mahaboob nagar district, Andhra Pradesh, India during the month of May. It was authenticated from Osmania University, Hyderabad.

Extraction of plant material

The root was shade dried and powdered coarsely. The coarse powder obtained was extracted exhaustively with 70% ethanol in soxhlet apparatus and filtered. The extract was concentrated under temperature and pressure to get dry residue and stored in a desicators. The same method has applied to seeds also.

6.2. Phytochemical screening of hydroalcoholic root extract

Preliminary Phytochemical analysis was carried to find out the Phytoconstituents present in crude extract. Crateva magna showed the presence of glycosides, tannins, saponins, terpenoids and flavonoids.

Test solution: The hydroalcoholic extract of *Crateva magna* was taken and dissolved in water.

6.3.a.ALKALOIDS

i) Dragendorff s test

To the extract, Dragendorff s reagent (potassium bismuth iodine solution) was added, reddish brown precipitate was produced.

ii) Mayer s test

To the extract, Mayer s reagent (potassium mercuric iodine solution) was added, cream colour precipitate was produced.

iii) Wagner s test

To the extract, Wagner s reagent (iodine-potassium iodide solution) was added, reddish brown precipitate was produced.

iv) Hagner s test

To the extract, Hagner s reagent (saturated solution of picric acid) was added, yellow precipitate was produced.

v) Tanic acid test

To the extract, tannic acid solution was added, buff colour precipitate was produced.

3.3.b. AMINOACIDS

i) Millon s test

To the test solution, about 2ml of Millon s reagent was added, a white precipitate was obtained indicating the presence of amino acid.

ii) Ninhydrin test

To the test solution, Ninhydrin solution was added, boiled, violet colour was produced indicating the presence of amino acid.

6.3.c. PROTEINS

i) Warming test

The test solution was heated in a boiling water bath, proteins got coagulated.

ii) Test with trichloroacetic acid

To the test solution trichloroacetic acid was added, precipitate was formed.

iii) Hydrolysis test

The test solution was hydrolysed with hydrochloric acid or sulphuric acid, Ninhydrin test was carried out for amino acids.

iv) Biuret test

To the test solution (2 ml), Biuret reagent (2 ml) was added, violet colour was produced indicating the presence of proteins.

v) Xanthoprotein test

To the (5 ml) of test solution, 1 ml of concentrated nitric acid was added and boiled, yellow precipitate was formed. After cooling it, 40% sodium hydroxide solution was added an orange colour was formed.

6.3.d. CARBOHYDRATES

i) Molish test

To the test solution, few drops of alcoholic α-napthol was added, then few drops of concentrated sulphuric acid was added through the sides of test tube, purple to violet colour ring appeared at the junction.

ii) Barfoed s test

1 ml of the test solution, was heated with 1 ml of Barfoed s reagent on water bath, if red cupric oxide was formed, monosaccharide was present.

iii) Selivanoff s test (Test of Ketones)

To the test solution, crystals of resorcinol and equal volume of concentrated hydrochloric acid were added and heated on a water bath, rose colour was produced.

iv) Test for pentoses

To the test solution, equal volume of hydrochloric small amount of phloroglucinol was added and heated, red colour was produced.

v) Fehling s test

To the test solution, equal quantity of Fehling s A and B were added and heated on water bath, brick red precipitate was formed.

vi) Benedict s test

To the test solution, 5ml of Benedict s reagent was added and heated on water bath, red precipitate was formed.

6.3.e. FLAVONOIDS

i) Shinoda test

To the test solution, few magnesium turnings were added and concentrated hydrochloric acid was added drop wise, pink scarlet, crimson red or occasionally green to blue colour appeared after few minutes.

ii) Alkaline reagent test

To the test solution, few drops of sodium hydroxide solution was added, intense yellow colour was formed which turned to colourless on addition of few drops of dilute acid indicating the presence of flavonoids.

iii) Zinc hydrochloride test

To the test solution, a mixture of zinc dust and concentrated hydrochloric acid were added. It gave red colour after few minutes.

6.3.f. Glycosides

i) General test

Test A

200 mg of drug was extracted with 5 ml of dilute sulphuric acid by warming on waterbath. It was filtered then the acid extract was neutralized with 5% solution of sodium hydroxide. 0.1 ml of Fehling s solution A and B was added until it became alkaline (tested with pH paper) and heated on water bath for 2 minutes. The quantity of red precipitate formed was noted and compared with that of formed in Test B.

Test B

200 mg of drug was extracted using 5 ml of water instead of dilute sulphuric acid. After boiling add equal amount of water and 0.1 ml of Fehling s solution A and B was added until it became alkaline (tested with pH paper) and heated on water bath for 2 minutes. The quantity of red precipitate formed was noted the quantity of precipitate formed in Test B was compared with that of formed in Test A.

The precipitate in Test A was greater than in Test B, showing the presence of glycoside.

6.3.g. Tannins (Phenolic compounds)

i) Ferric chloride test

To the test solution, ferric chloride solution was added, green colour appeared showing the presence of condensed tannins.

ii) Phenazone test

To the test solution, 0.5 grams of sodium acid phosphate was added, warmed and filtered. To the filtrate add 2% phenazone solution, bulky precipitate was formed which was often coloured.

iii) Gelatin test

To the test solution, 1% gelatine solution containing 10% sodium chloride was added. Precipitate was formed.

iv) Test for catechin

Matchstick was dipped in the test solution, dried it and lastly moistened with concentrated hydrochloric acid. Then the stick was warmed near to flame. The colour of the wood changed to pink due to phloroglucinol (Phloroglucinol was fomed when catechins were treated with acids).

6.3.h. STEROIDS AND TERPENOIDS

i) Libermann-Burchard test

The extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction of two layers and upper layer turned green which showed the presence of steroids and formation of deep red colour indicating the presence of triterpenoids.

ii) Salkowski test

The extract was treated with few drops of concentrated sulphuric acid, red colour at lower layer indicates the presence of steroids and formation of yellow coloured lower layer indicating the presence of triterpenoids.

The same method of extraction has been done for seeds of Eugenia jambolana.

6.4 ACUTE TOXICITY STUDY

1. *Crateva magna*

Whenever an investigator administers a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved be lethal to 50% of the tested group of animals.)

Determination of oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. This article reviews the methods so far utilized for the determination of median lethal dose (LD_{50}) and the new different categories of substances before its final acceptance by regulatory bodies.

Organisation of economic co-operation and development (OECD) regulates guidelines for acute oral toxicity. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. To determine the acute toxicity OECD frames the following guideline methods.

OECD 401 - Acute oral toxicity

OECD 420 - Acute oral toxicity : fixed dose procedure

OECD 423 - Acute oral toxicity : Acute toxic classic method

OECD 425 - Acute oral toxicity : Up and down procedure (UDP)

In the present study, the acute oral study of Actinomers dichotoma Bedd. was carried out according to OECD 423 guideline (Acute oral toxicity : Toxic classic method).

Acute oral toxicity:

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hrs.

LD_{50} (median lethal dose):

LD_{50} statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Principle

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute study of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.,

- No further testing is needed.
- Dosing of three additional animals, with the same dose.
- Dosing of three additional animals at the next higher or the next lower dose level.

Selection of animal species

The preferred rodent species was the rat. Normally females were used. Females were generally slightly more sensitive. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing should be between 8 to 12 weeks old.

Administration of doses

The test substance was administered in a single dose by gavage using an oral feeding needle. Animals should be fasted to dosing (e.g. with the rat food but not water should be withheld over-weight with the mouse food but not water should within for 3-4 hrs). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be with held for a further 3-4 hrs in rats.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours with the special attention given during the first 4 hrs and daily thereafter, for a total of 14 days except where they need to be removed from the study and humanely killed for animal welfare reasons or found dead. However, the duration of observation

should not be fixed rigidity. It should be determined by the toxic reactions time of onset and length of recovery period and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Dose selection

According to OECD 423 guideline dose was selected on the basis of maximum tolerable dose (NOAEL), as there was no lethal dose observed upto 3000mg/kg. Thus dose was selected as $1/10^{th}$ of 3000mg/kg, i.e., 300mg/kg was taken as high dose and 100mg/kg was taken as low dose.

Same procedure was adopted for Eugenia jambolana and findings given in the results.

6.5. Evaluation of Anti diabetic, Hypolipidemic activity

6.5.1. Animals and treatment schedule:

Thirty male rats, 6 weeks old and weighing roughly above 150g, were procured from Nishka Labs and were housed in polupropylene cages in a temperature controlled room (25±2˚C) witha 12h light/12h dark cycle. All rats were adapted and fed a standard pellet diet for 1 week. Rats had unrestricted access to food and water. Food intakes were monitored daily, and body weights measured weekly. All rats were randomly divided into five groups (n=6),

Group I:- Normal control group.

Group II:- Diabetic control group fed with STZ.

Group III:- STZ treated group with standard drug Glibenclamide (10mg/kg;p.o).

Group IV:- STZ treated group with Crateva magna root extract 100mg/kg;p.o.

Group V:- STZ treated group with Crateva magna root extract 300mg/kg;p.o.

All the above groups were treated for 21 days and rats were maintained in accordance with the National Institute of nutrition, Indian Council of Medical Research, Hyderabad, India guidelines for the care and use of laboratory animals.

The same procedure was followed for Eugenia jambolana seed and 1:1 combination of Carteva magna root and Eugenia jambolana seed.

6.5.2. Blood collection techniques used in the present study

Blood and tissue collection:

At the end of the study, blood samples were collected in the heparinized tube by puncturing the orbital venous plexus of 12h fasted and anaesthetized (slight exposure to ether) rats. Whole blood samples were centrifuged at 4500 rpm for 10 min at 4˚C and plasma was separated out and stored at -70˚C until further analysis. All the animals were sacrificed by cervical dislocation, liver, pancreas were removed and rinsed with chilled phosphate buffered saline (pH 7.0) and weighed.

Picture no: 5. Retro-orbital bleeding technique

6.5.3. ESTIMATION OF BIOCHEMICAL PARAMETERS

The biochemical parameters was estimated by using commercially available kits, according to the manufacturer protocol, the following parameters GLUCOSE(by accu-check active) total cholesterol, triglycerides, HDL, LDL, VLDL were estimated by using auto in NISHKA LABS.

6.5.3.a. Estimation of glucose

The glucose levels are estimated by collecting the blood samples. Small amount of blood was obtained from tail vein of rat. By placing a drop of blood on glucometer strip there by the strip was inserted into the glucometer where it displaces the glucose level in the drop of blood in mg/dl.

6.5.3.b. Estimation of total cholesterol

Method Cholesterol oxidase-peroxidase

Principle

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction, cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidise, hydrogen peroxidise oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye which has absorbance maximum at 510 nm. The intensity of the red colour is proportional to the amount of total cholesterol in the specimen.

Cholesterol esters ?????????????? cholesterol + fatty acids.

Cholesterol + O_2 ?????????????? H_2O_2 + cholest-4-en-3-one.

$2H_2O_2$ + 4-aminoantipyrine ??????? red quinoneimine dye + H_2O_2 + phenol.

Reagent composition

S.NO	REAGENT	CONCENTRATION
1	Buffer, pH 6.8	50mMol/lit
2	Cholesterol oxidase	≥100IU/L
3	Cholesterol esterase	≥150IU/L
4	Peroxidase	≥500IU/L
5	4-amino antipyrine	0.5mMol/lit
6	Phenol	≥10mMol/lit
7	Stabilizer/surfactant	Qs

Assay parameters

Reaction type - end point	Linearity - 1000 mg/dl
Reagent volume - 1 ml (1000 µl)	Zero setting with - reagent blank
Wavelength - 510 nm	Sample volume - 0.01 ml (10 µl)
Blank absorbance limit - 0.300 Abs	Standard concentration - 200 mg/dl
Reaction time - 5 minutes at 37˚C/10 minutes at room temperature (25˚-30˚C)	

Procedure

Sample - serum

The required amount of reagent before use was pre warmed at room temperature, the assay was performed as given below.

	Serum	**Standard**	**Blank**
	10 µL	10 µL	-
Reagent	1000 µL	1000 µL	1000 µL

Incubation

The assay mixture was incubated for 5 minutes at 37˚C for 10 minutes at room temperature (25˚-30˚C). After incubation the absorbance of the assay mixture was measured against blank at 510 nm. The final colour was stable for two hours if not exposed to direct light.

Calculation

Total cholesterol (mg/dL) = (absorbance of test/absorbance of standard) × 200

Estimation of triglycerides

6.5.3.c. Triglycerides

Method Glycerol 3-phosphate oxidase- GPO-PAP METHOD

Principle

Triglycerides are hydrolysed by lipoprotein lipase (LPL) to produce Glycerol and free fatty acid (FFA). In presence of glycerol kinase (GK), adenosine tri phosphate (ATP) phosphorylates glycerol to produce glycerol 3- phosphate and adenosine diphosphate (ADP). Glycerol 3- phosphate is further oxidized by glycerol 3-phophate oxidase (GPO) to produce dihydroxyacetone phosphate (DAP) and H_2O_2. In thepresence of peroxidise (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and 4-chlorophenol to produce red quinoneimine dye. Absorbance of the coloured dye is measured at 505 nm and is proportional to triglycerides concentration in the sample.

Triglyceride ?——————? glycerol + free fatty acids

Glycerol + ATP ?——————? Glycerol 3-phosphate + ADP

Glycerol 3-phosphate ?——————————? dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + 4-chlorophenol?———? quinoneimine dye + $4H_2O_2$

Reagent composition

Reagent No.	Reagent	Composition	Concentration
1	Triglyceride mono reagent	Pipers buffer, (PH 7) Mg ions p-Chlorophenol ATP Potassium ferrocyanide Amino-4-antipyrine Lipoprotein lipase Glycerol kinase Glycerol-3-phosphate oxidase Peroxidase Detergents, preservatives, stabilizers	50 mmol/L 14.8 mmol/L 2.7 mmol/L 3.15 mmol/L 10 mmol/L 0.31 mmol/L ≥2000 U/L ≥500 U/L ≥4000 U/L ≥500 U/L Qs
2	Triglyceride standard	Glycerol (Triglycerides equivalent) Preservatives, stabilizers	200 mg/Dl Qs

Assay parameters

Mode - end point	Wavelength - 505 nm
Flow-cell - 37˚C	Optical path length - 1 cm
Blanking - reagent blank	Reagent volume - 1000 µL
Sample volume - 10 µL	Incubation time - 10 minutes at 37˚C
Concentration of std - 200 mg/dL	Stability of colour - 1 hour
Linearity - 1000 mg/dL	Permisible - <0.3 AU

Procedure

Sample - serum

Samples Pipette into tube marked	**Blank**	**Standard**	**Test**
Serum	-	-	10 µL
Reagent 2	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL

The serum was mixed well. Incubated at 37˚C for 10 minutes.

The analyser was programmed as per assay parameters.

1. The analyser was blanked with reagent blank.
2. The absorbance of standard followed by the test was measured.
3. The results were calculated as per given formula.

Serum triglycerides (mg/dL) = (absorbance of test/absorbance of standard) × 200

6.5.3.d. HDL

Method-Polyethylene Glycol-CHOD-PAP with LCF (lipid clearing factor)

Principle

Low and very low density lipoproteins (VLDL) are precipitated by a solution containing PEG 6000, leaving behind the high density lipoproteins in the solution, HDL cholesterol is estimated in the supernatant by a series of enzymatic reactions which are initiated by the oxidation of cholesterol to cholestenone by cholesterol oxidase, accompanied by the formation of hydrogen peroxide. In a second reaction catalysed by peroxidase, 4-aminoantipyrine and phenol react with hydrogen peroxide to form red colour quinoneimine. Asorbance at 505 nm is directly proportional to HDL cholesterol concentration in the specimen.

Reagent compositions

Precipitating reagent(Reagent 3);PEG 6000; Stabilizer; Preservative; HDL-cholesterol standard 50 mg/dL (Reagent 4). Cholesterol; Stabilizer; Preservative.

Assay parameters

Mode - end point	Wavelength - 505 nm
Flow-cell - 37˚C	Optical path length - 1 cm
Blanking - reagent blank	Reagent volume - 1000 µL
Sample volume - 100 µL	Stability of colour - 1 hour
Concentration of standard - 50 mg/dL	Maximum absorance limit - 2.0
Incubation time - 10 minutes at 37˚C/30 minutes at room temperature (15-30˚C)	

Procedure

Sample-serum

Step A: HDL cholesterol separation

Samples pipette into centrifuge tube	**Quantity**
Sample	200 µL
Precipitating reagent	200 µL

The serum was mixed well, kept at room temperature (15-30˚C) for 10 minutes and then centrifuged at 2000 rpm for 15 minutes to obtain a clear supernatant.

Step B: Colour development.

Samples pipette into tube marked	**Blank**	**Standard**	**Test**
Supernatant from step A	-		100 µL
HDL cholesterol standard	-	100 µL	-
Cholesterol reagent	1000 µL	1000 µL	1000 µL

Mixed well, incubated at 37˚C for 10 minutes or at room temperature (15-30˚C) for 30 minutes. The absorbance was read against blank at 505 nm within 60 minutes.

Calculation:

HDL cholesterol (mg/dL) = (absorbance of test/absorbance of standard) × 50 × 2

6.5.3.e. VLDL

The VLDL was calculated by using the following formula

VLDL (mg/dl) = (Triglyceride/5)

6.5.3.f. LDL

The LDL was calculated by using the following formula

LDL (mg/dl) = Total cholesterol - (HDL + VLDL)

ORAL GLUCOSE TOLERANCE TEST

A singular feature of Diabetes mellitus is impaired glucose tolerance. This is unmasked by an oral glucose tolerance test, in which blood glucose levels are sampled after overnight and then minutes to hours after an oral dose of glucose. In normal persons blood glucose levels rise only modestly, and a brisk insulin pancreatic insulin response ensures a return to normoglycemic levels within an hour. In diabetic individuals and those in preclinical stage, blood glucose rises to abnormally high levels for a sustained period. This may result from an absolutely lack of pancreatic insulin release or from impaired target tissue response to insulin or both.

Procedure:

OGTT is carried out in the diabetic induced rats. The rats were divided into 5 groups consisting of 6 animals in each group.

Group I:- Norma control received 0.9% saline.

Group II:- Diabetic control received 0.9% saline.

Group III:- Diabetic rats treated with 10mg/kg of std glibenclamide

Group IV:- received HAECMR (100mg/kg;p.o) + glucose (2gm/kg;p.o)

Group V:- received HAECMR (300mg/kg;p.o) + glucose (2gm/kg;p.o)

After 60 min of drug administration, the rats of normal and diabetic groups were orally treated with glucose (2gm/kg;p.o). The blood samples were drawn from the tail vein at 0, 30, 60, 90 and 120 min after glucose feeding. Glucose levels were determined using an Accu-Check Advantage Blood Glucose Monitor (Roche Group, Indianapolis, IN, USA).

EXPERIMENTAL DESIGN

Five groups of rats, six in each group received the following treatment schedule for 21 days.

GROUP I:- normal control.

GROUP II:- STZ treated control.

GROUP III:- STZ + Glibenclamide (10mg/kg;p.o).

GROUP IV:- STZ + HAECMR (100mg/kg;p.o).

GROUP V:- STZ + HAECMR (300mg/kg;p.o).

Root extract of *Crateva magna*, standard drug were administered with the help of oral feeding needle.

STZ- Streptozotocin.

HAECMR- Hydroalcoholic extract of *Crateva magna* root.

Fasting blood samples were drawn from retro orbital venous plexus of rats at 4 intervals (each interval = 7 days) for 21 days till the end of the study 0, 7, 14, 21 days.

ESTIMATION OF BIOCHEMICAL PARAMETERS

On 0, 7, 14, 21 days fasting blood samples were collected, serum was separated and analysed for glucose, 1, 12, 24,36 and 48^{th} days total cholesterol, triglycerides, HDL, VLDL, LDL and plasma insulin levels.

EVALUATION OF WEIGHT OF BODY

Body weights of all the animals in each group was measured during the study period initially before the start of the study and finally at the end of the study.

STATISTICAL ANALYSIS

Statistical analysis was done by using GRAPHPAD PRISM 5.0.

All the values of OGTT, biochemical parameters, and body weight were expressed as Mean ± Standard Error Mean (S.E.M.). The values were analyzed for statistically significance using one-way analysis of variance (ANOVA), followed by Dunnett s tests.

7. RESULTS AND DISCUSSION

7.1 RESULTS

7.1.1. Appearance and percentage yield of HAECMR

The colour consistency of HAECMR was noted and the percentage yield was calculated and reported.

Colour - brownish

Consistency - semisolid

Percentage yield - 10.12%

7.1.1.a. Preliminary phytochemical screening:

Table no: 4. Results of the preliminary phytochemical constituents present in HAECMR

S.NO	Plant constituents	HAECMR
1	Alkaloids	+ve
2	Aminoacids	+ve
3	Proteins	+ve
4	Carbohydrates	+ve
5	Flavonoids	+ve
6	Glycosides	+ve
7	Tanins	+ve
8	Terpenoids	+ve
9	Saponins	+ve
10	Phenolic	+ve

+ve indicates present and -ve indicates absent

RESULTS:

The phytochemicals present in the hydroalcoholic extract of *Crateva magna* were aminoacids, carbohydrates, flavonoids, glycosides, Tanins, terpenoids, saponins, phenolic compounds.

7.1.2.ACUTE TOXICITY STUDY

OCED 423- Acute oral toxicity : Toxic classic method

The effect of different doses of hydro alcoholic extract of *Crateva magna* root on acute toxicity test.

Table no: 5. Results of the percentage mortality, signs of toxicity of different doses of HAECMR treated groups

Group (n=3)	Treatment	Mortality at 14^{th} day	% Mortality	Signs of toxicity
1	Normal control	0/3	0	-
2	HAECMR 300 mg/kg; p.o.,	0/3	0	-
3	HAECMR 2000 mg/kg; p.o.,	0/3	0	-
4	HAECMR 3000 mg/kg; p.o.,	0/3	0	-

+ indicates presence and - ve indicates absence

RESULTS

The percentage mortality signs of different doses of HAECMR treated groups was shown in the table.

The different doses of hydroalcoholic extract of *Crateva magna* root treated rats showed 0% mortality and absence of toxicity. This revealed the non-toxic nature of the hydroalcoholic extract of *Crateva magna* root even at high dose of 3000mg/kg body weight.

7.1.3. ORAL GLUCOSE TOLERANCE TEST (OGTT)

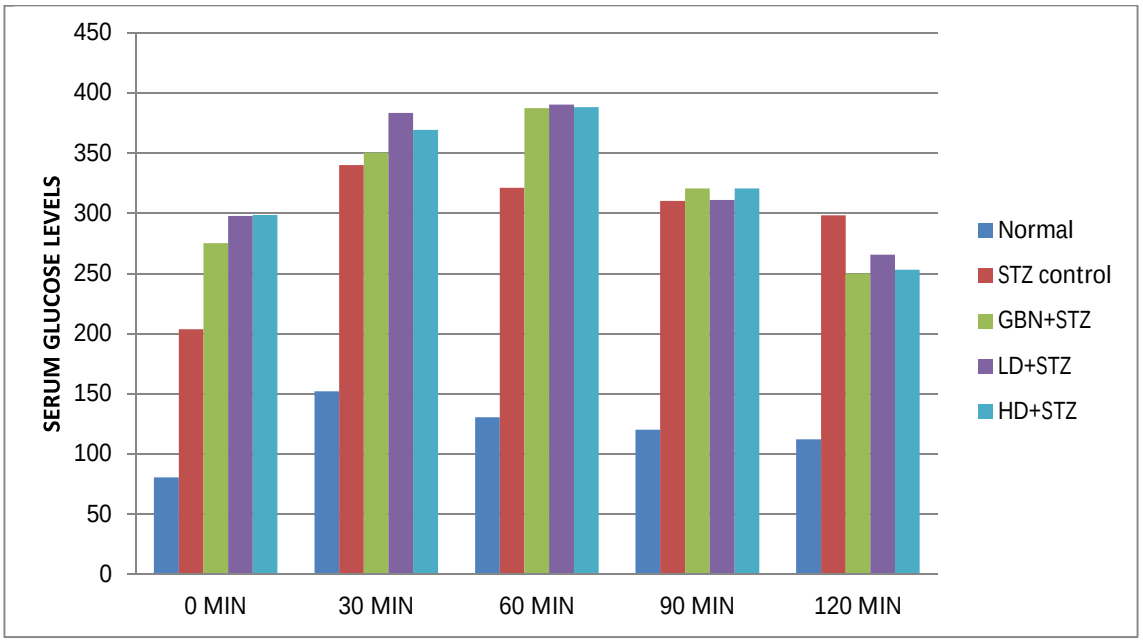
The effect of different doses of Hydroalcoholic extract of *Crateva magna* root on oral glucose tolerance test in normal rats.

Table no: 6. Results of effects of HAECMR on OGTT

Group	Treatment	SERUM GLUCOSE (mg/ml)				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
1	Normal control	80.56± 1.68	152.20± 1.25	130.70± 1.12	120.1± 1.62	112.37± 2.21
2	STZ treated diabetic control	203.71± 2.52	330.21± 2.51	321.43± 2.77	310.44± 3.63*	298.51± 3.14
3	Glibenclamide(10mg/kg;p.o) + glucose (2 gm/kg;p.o)	275.43± 7.64	350.63± 7.31	387.50± 7.37	320.71± 6.46	250.31± 8.34
4	HAECMR (100 mg/kg;p.o) + glucose (2 gm/kg;p.o)	298.86± 12.31	383.56± 5.08	390.5± 7.13**	311.40± 6.92	265.73± 5.96**
5	HAECMR (300 mg/kg;p.o) + glucose (2 gm/kg;p.o)	298.88± 13.52	369.57± 8.10	388.51± 7.24	320.71± 6.20**	253.31± 9.82**

The values were estimated as Mean+S.E.M (n=6). Extract treated, Glibenclamide treated and glucose treated were compared with the vehicle control and STZ treated. **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.

Graph no: 1. Diagramatic representation of results of the effects of HAECMR on OGTT



GBN-Glibenclamide(10mg/kg;p.o), GLU-Glucose(2gm/kg;p.o), HD-High dose(300mg/kg;p.o) of extract, LD-low dose(100mg/kg;p.o), STZ-Streptozotocin

Results

In Glucose Tolerance Test, the Glucose levels were estimated at 0,30,60,90 and 120 Mins respectively. The blood sugar level of extract treated groups were found compared to be diabetic control and the effects were dose dependant. Group II & IV glucose lowering efficiency between 90-120 Mins & were comparable to diabetic standard shown in table.

The experiment showed that OGTT measures the ability to use glucose, the bodys main source of energy, used to diagnose pre & post diabetes, glucose lowering effects were found after oral administration of extracts in rats may be due to the presence of hypoglycaemic flavanoids, terpenes or saponins that also requiring further investigation.

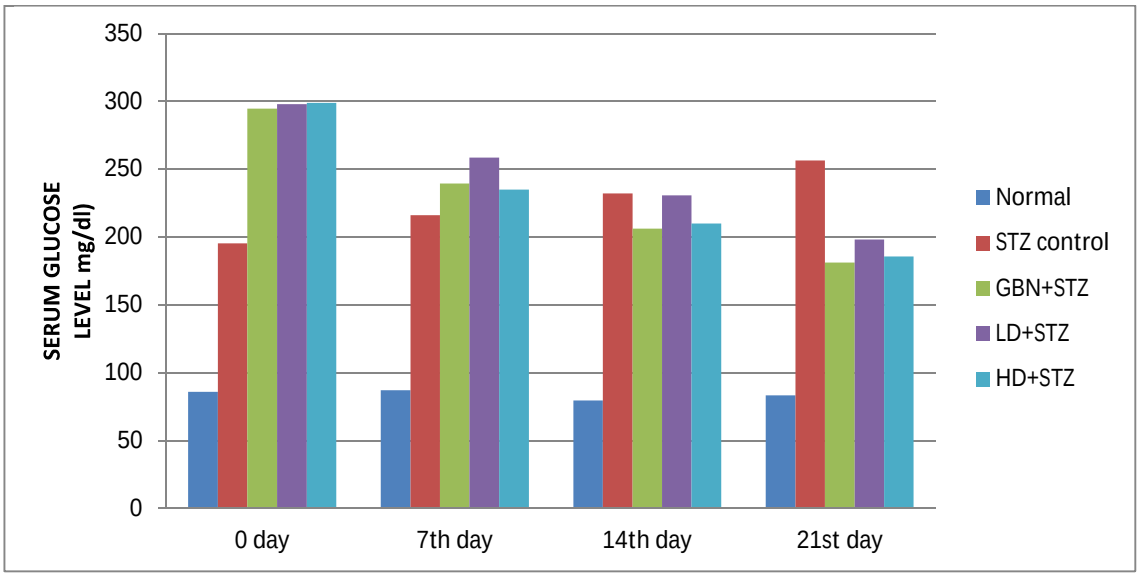
7.1.4. ESTIMATION OF BIOCHEMICAL PARAMETERS

7.1.4.a. Estimation of blood glucose

Table no: 7. Results of the effects of HAECMR on serum glucose levels

Group	Treatment	Serum glucose levels (mg/dl)			
		0st day	**7th day**	**14th day**	**21st day**
1	Normal control	85.81± 5.52	87.21±5.36	79.59±5.64	83.45±5.72
2	STZ treated diabetic control	195.47± 6.33***	216.25±8.36***	232.21±10.299***	256.40±8.65***
3	Glibenclamide(10 mg/kg;p.o) + STZ	294.71± 12.04	239.45±5.71**	206.21±7.74**	181.21±7.22**
4	HAECMR (100 mg/kg;p.o) + STZ	298.01±12.31**	258.63±7.33***	230.52±11.30**	198.20±9.63**
5	HAECMR (300 mg/kg;p.o) + STZ	298.88± 13.52	235.11±5.54***	210.03±3.35**	185.71±7.84***

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 2. Diagramatic representation of results of the effects of the HAECMR on Serum glucose levels

STZ- Streptozotocin control,

GBN- Glibenclamide(10mg/kg;p.o)

LD-low dose(100mg/kg;p.o) of extract

HD-high dose(300mg/kg;p.o) of extract.

RESULTS

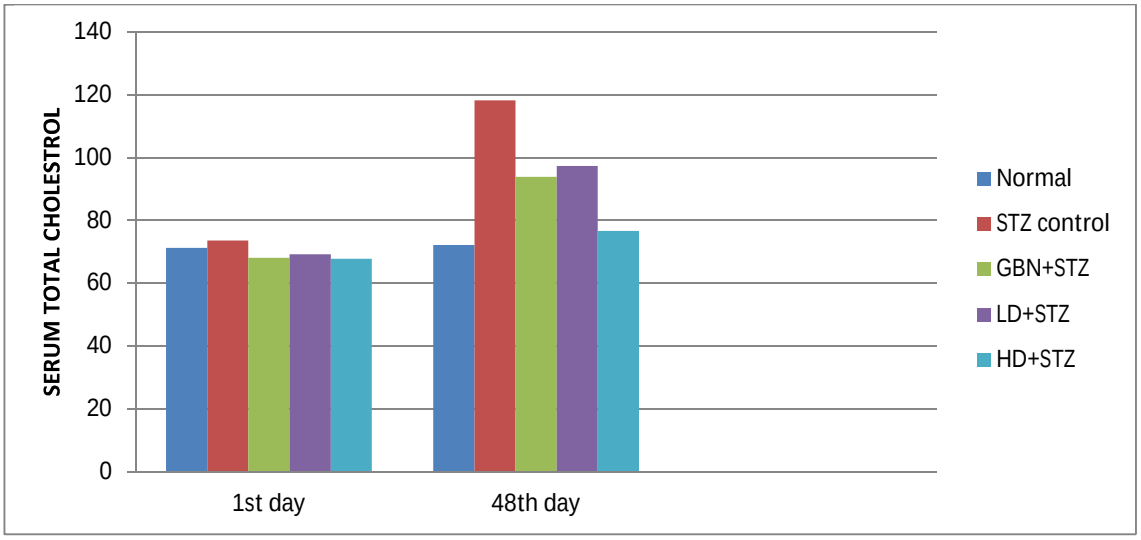
The blood glucose levels were measured on 0, 7^{th}, 14^{th}, 21^{st} days were showed in Table no. 7. When compared with normal control, STZ treated diabetic control group on 0, 7^{th}, 14^{th} and 21^{st} days showed significant ($p<0.001$) values as compared to STZ treated diabetic control group. Glibenclamide(10mg/kg;p.o)+STZ treated diabetic group showed significant decrease in blood glucose levels on 7^{th}, 14^{th}, 21^{st} day($p<0.05$). When compared to STZ treated diabetic control group HAECMR (300mg/kg;p.o) showed significant decrease in blood glucose levels($p<0.05$) and its effect is almost comparable to that of Glibenclamide.

7.1.4.b ESTIMATION OF TOTAL CHOLESTEROL

Table no: 8. Results of the effects of HAECMR on serum total cholesterol levels

Group	Treatment	Serum total cholesterol (mg/dl)	
		1st day	**48th day**
1	Normal control	71.28±5.23	72.18±7.09
2	STZ treated control	73.63±7.35	118.30±16.19
3	Glibenclamide (10 mg/kg;p.o) + STZ	68.15±3.12	93.93±10.2
4	HAECMR (100 mg/kg;p.o) + STZ	69.16±6.12	97.38±7.32
5	HAECMR (300 mg/kg;p.o) + STZ	67.79±4.14	76.7±4.78*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 3. Diagramatic representation of the results of the effects of the HAECMR on total cholesterol levels

STZ- Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

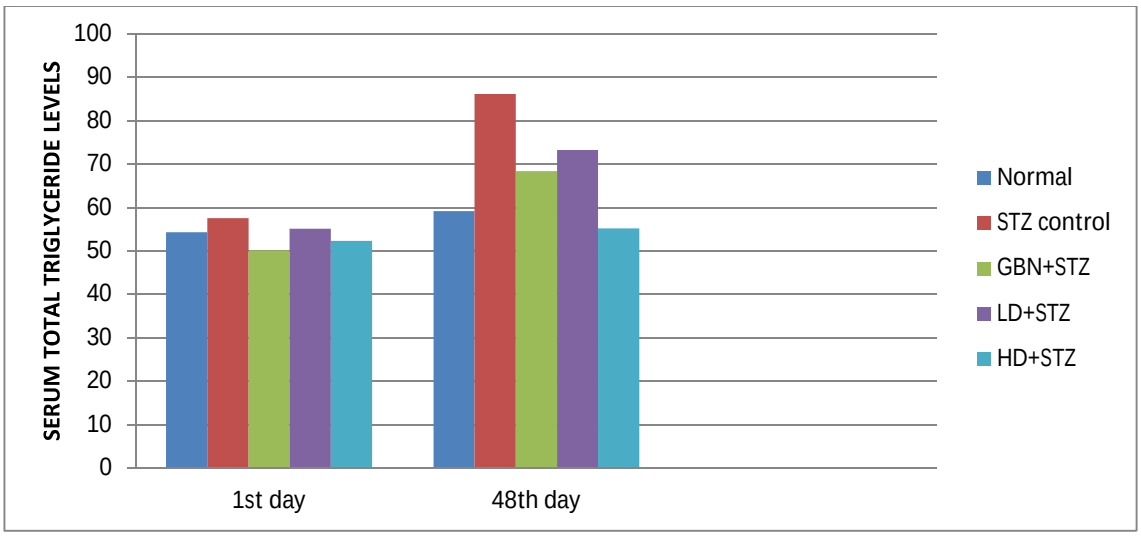
When compared with the STZ treated diabetic control group, Glibenclamide+STZ treated group showed significant decrease in total cholesterol levels($p<0.05$). When compared with STZ treated diabetic group control group and Glibenclamide+STZ treated group HAECMR (300mg/kg;p.o) group showed significant decrease in total cholesterol levels($p<0.05$).

7.1.4.c. ESTIMATION OF TRIGLYCERIDES

Table no: 9. Results of the effects of HAECMR on serum triglyceride levels

Group	Treatment	Serum triglycerides (mg/dl)	
		1st day	**48th day**
1	Normal control	54.27±3.71	59.20±4.33
2	STZ treated control	57.56±3.16	86.15±2.89
3	Glibenclamide(10mg/kg;p.o) + STZ	50.09±2.81	68.39±3.98
4	HAECMR (100 mg/kg;p.o) + STZ	55.12±7.1	73.29±8.0
5	HAECMR (300 mg/kg;p.o) + STZ	52.33±5.12	55.2±3.41*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 4. Diagramatic representation of the results of the effects of the HAECMR on TG levels

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

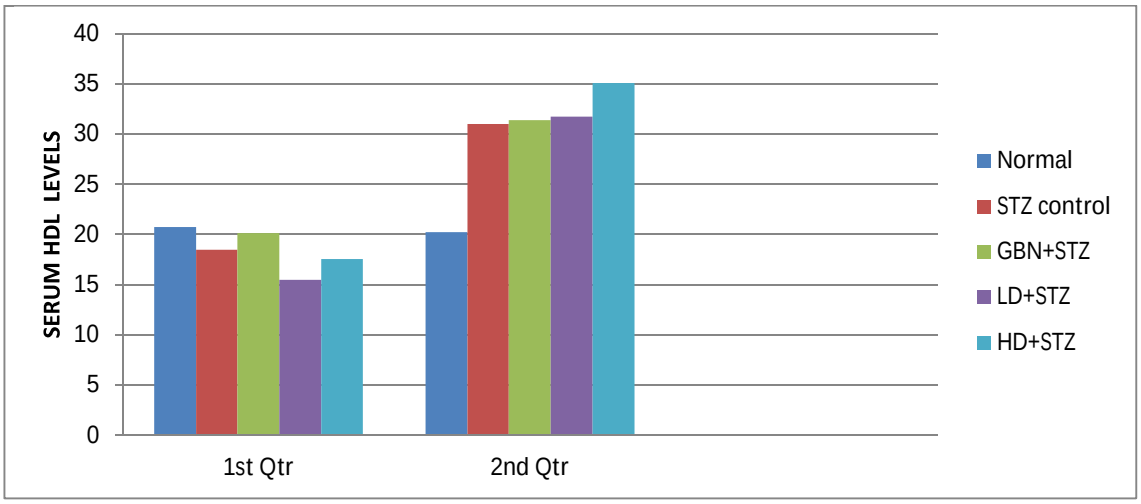
When compared with the STZ treated diabetic control group, Glibenclamide+STZ treated group showed significant decrease in Serum triglycerides level($p<0.05$). When compared with STZ treated diabetic control group and Glibenclamide+STZ treated group HAECMR (300mg/kg;p.o) showed significant decrease in Serum triglycerides level($p<0.05$).

7.1.4.d. ESTIMATION OF HDL

Table no: 10. Results of the effects of HAECMR on serum HDL levels

Group	Treatment	Serum HDL (mg/dl)	
		1st day	**48th day**
1	Normal control	20.72±3.56	20.23±2.68
2	STZ treated control	18.49±4.16	31.01±3.51
3	Glibenclamide(10mg/kg;p.o) + STZ	20.14±3.69	31.35±2.76
4	HAECMR (100 mg/kg;p.o) + STZ	15.49±4.73	31.73±2.89
5	HAECMR (300 mg/kg;p.o) + STZ	17.57±3.84	35.06±3.16*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 5. Diagramatic representation of results of HAECMR on HDL

STZ-Steptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

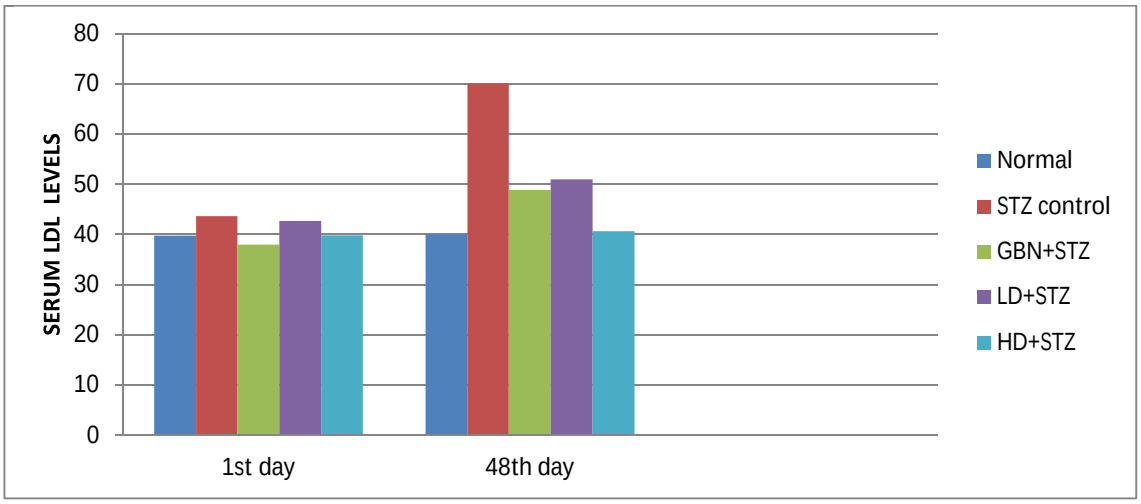
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAECMR (300mg/kg;p.o) showed significant increase in HDL levels($p<0.05$).

7.1.4.e. ESTIMATION OF LDL

Table no: 11. Results of the effects of HAECMR on serum LDL levels

Group	Treatment	Serum LDL (mg/dl)	
		1st day	**48th day**
1	Normal control	39.71±5.24	40.11±3.81
2	STZ treated control	43.63±4.68	70.06±12.0
3	Glibenclamide(10mg/kg;p.o) + STZ	38.00±5.11	48.91±8.30
4	HAECMR (100 mg/kg;p.o) + STZ	42.65±2.48	51.0±3.53
5	HAECMR (300 mg/kg;p.o) + STZ	39.76±2.64	40.6±0.04*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 6. Diagramatic representation of results of effects of HAECMR **on LDL levels**

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

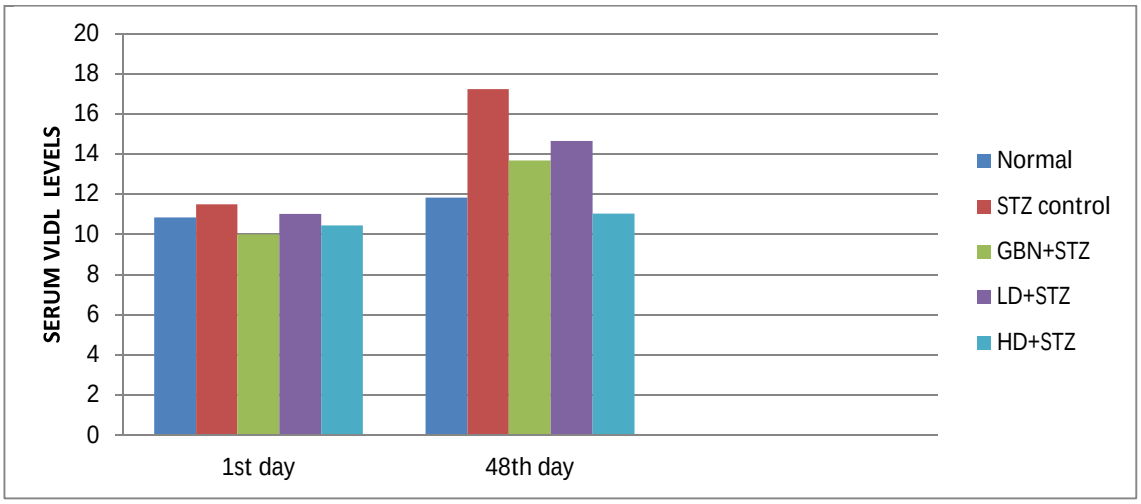
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAECMR (300mg/kg;p.o) showed significant decrease in LDL levels.

7.1.4.f. ESTIMATION OF VLDL

Table no: 12. Results of the effects of HAECMR on serum VLDL levels

Group	Treatment	Serum VLDL (mg/dl)	
		1st day	**48th day**
1	Normal control	10.85±0.74	11.84±0.86
2	STZ treated control	11.51±0.63	17.23±0.57
3	Glibenclamide (10mg/kg;p.o) + STZ	10.01±0.56	13.67±0.57
4	HAECMR (100 mg/kg;p.o) + STZ	11.02±1.42	14.65±1.6
5	HAECMR (300 mg/kg;p.o) + STZ	10.46±1.02	11.04±0.68*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 7. Diagramatic representation of results of effects of HAECMR on VLDL levels

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-low dose(100mg/kg;p.o) of extract, HD-high dose(300mg/kg;p.o) of extract.

RESULTS

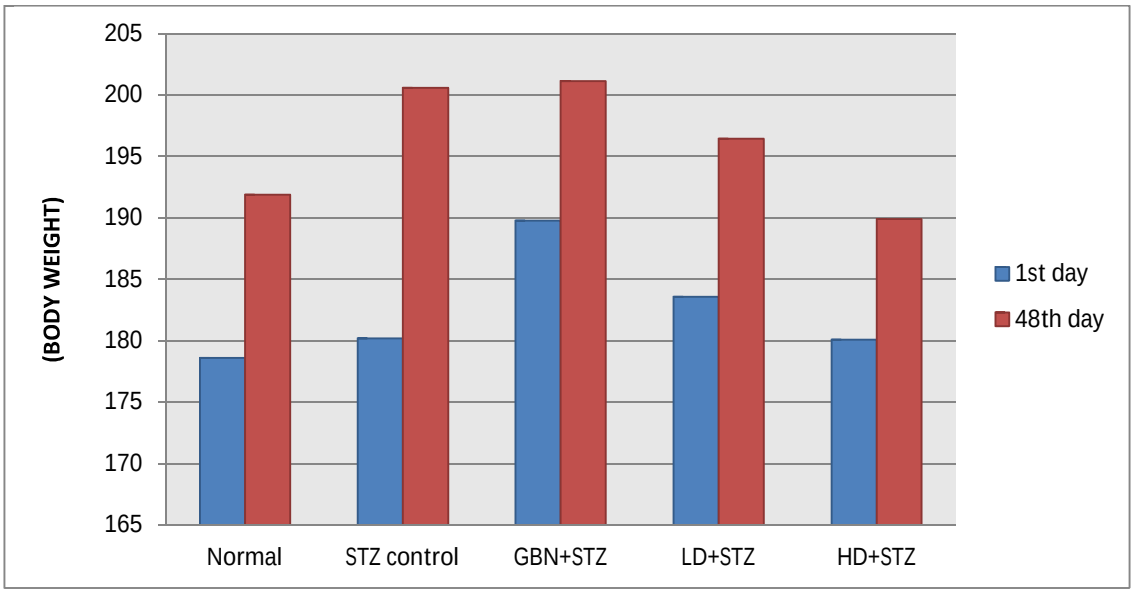
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAECMR (300mg/kg;p.o) showed significant decrease in VLDL levels($p<0.05$).

7.1.4.g. ESTIMATION OF BODY WEIGHT

Table no: 13. Results of the effects of HAECMR on body weight

Group	Treatment	Body weight (gm)		
		0^{th} day	**48^{th} day**	**Gain**
1	Normal control	178.60±5.7	191.9±5.1	13.30±4.50
2	STZ treated control	180.19±3.32	200.57±4.13	20.38±5.0
3	Glibenclamide (60 µg/kg;p.o) + STZ	189.75±5.1	201.13±5.0	11.38±4.98
4	HAECMR (100 mg/kg;p.o) + STZ	183.56±4.2	196.45±3.9	12.89±2.9
5	HAECMR (300 mg/kg;p.o) + STZ	180.10±3.9	189.9±7.05*	9.8±4.6*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the normal control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 8. Diagramatic representation of results of effects of HAECMR on body weight before and after treatment of standard and HAECMR

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

When compared with STZ treated diabetic control group, Glibenclamide+STZ group and HAECMR (300mg/kg;p.o) showed significant difference in the body weight($p<0.05$).

7.2. DISCUSSION

The management of diabetes without any side effect is still a challenge to the medical system. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. Wide array of plants have demonstrated anti diabetic activity. The main active constituents of these plants include alkaloids, terpenoids, Tanins, saponins, flavonoids, proteins, gum, carbohydrates, phenolic compounds, terpenoids, amino acids and inorganic ions. These affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body.[8]

The colour consistency of the hydroalcoholic extract of Crateva magna was noted and the percentage yield was calculated.

Colour - Brownish green

Consistency - Semisolid

Percentage yield - 10.12 %w/w.

The preliminary phytochemical analysis bark of *Crateva magna* Root extract showed the presence of alkaloids, amino acids, proteins, carbohydrates, flavonoids, glycosides, Tanins, terpenoids, saponins, phenolic compounds.

Acute toxicity studies revealed the non-toxic nature of the Hydroalcoholic extract of *Crateva magna*. There was no lethality or any toxic reactions found with high dose (3000 mg/kg) till the end of the study. According to the OECD 423 guideline (Acute Oral Toxicity: Acute Classic Method), an LD_{50} dose of 3000 mg/kg and above is consider as unclassified so the hydroalcoholic extract of Crateva magna root was found to be safe.

A singular feature of diabetes mellitus is impaired glucose tolerance. This is unmasked by an oral glucose tolerance test, in which blood glucose levels are sampled after overnight fasting, and then minutes to hours after an oral dose of glucose. In normal persons, blood glucose levels rise only modestly, and a brisk pancreatic insulin response ensures a return to normoglycemic levels within an hour. In diabetic individuals and in those in a preclinical stage, blood glucose rises to abnormally high levels for a sustained period. This may result from an absolute lack of pancreatic insulin release or from impaired target tissue response or both.

The results of glucose intolerance induced by Streptozotocin and comparative delaying effect of *Crateva magna* as well as Glibenclamide in rats during 6 weeks of the experimental period. In Glucose Tolerance Test, the Glucose levels were estimated before drug treatment and at different intervals thereafter. In the control group, the blood glucose was found to increase linearly from basal value of 80.56 mg/dl to 152.20mg/dl in the first 30 minutes. After 60 minutes of glucose loading, the blood glucose was increased further. The maximum value of 112.37 mg/dl was seen at the 120^{th} minute. Whereas in the extract treated animals, only a little elevation in the blood glucose were seen from basal value of 298.88 mg/dl to 369.57 mg/dl in the first 30 minutes, 388.5 mg/dl in the 60 minutes and 320.7 mg/dl at 90 minutes and maximum glucose tolerance was observed at 120^{th} minute. In Glibenclamide treated animals, blood glucose rises to 350.63 mg/dl from basal value of 275.43 mg/dl in the first 30 minute, 387.5 mg/dl in 60 minutes and 250.31 mg/dl at 90 minutes and maximum glucose tolerance was observed at 120^{th} minute.

Mechanisms involved in the development of type 2 diabetes in models of Streptozotocin induced Diabetes in adult rodents are increased dietary fat intake is associated with reduced activity of pancreatic enzymes, leading to impaired pancreatic function and reduced insulin secretion and increased accumulation of lipids in the liver (hepatic stenosis) which results in hepatic insulin resistance and increased hepatic glucose output.

The exposure of the liver to large quantities of Streptozotocin leads to rapid stimulation of lipogenesis and triglycerides accumulation which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. The long-term negative effects can include changes in digestion, absorption, plasma hormone levels, appetite, and hepatic metabolism leading to the development of insulin resistance , diabetes, obesity, and inevitably cardiovascular disease. Because of its lipogenic properties, Streptozotocin can cause glucose malabsorption, together with greater elevations in triglycerides and cholesterol compared to other carbohydrates.

At the end of the study (48^{th} day) the hydroalcoholic extracts of *Crateva magna* root (100 mg/kg;p.o & 300 mg/kg;p.o) and Glibenclamide (10mg/kg;p.o) treated diabetic groups showed statistically significant decrease in blood glucose. So the hydroalcoholic extracts of *Crateva magna* root showed the anti diabetic activity.

In, diabetes hyperglycemia is accompanied with dyslipidemia represents risk factor for coronary heart diseases. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to action of insulin. Under normal

circumstances, insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not associated due to insulin deficiency resulting in hypertriglyceridemia and also associated with hyperchlosterolemia due to metabolic abnormalities. The dyslipidemia is characterized by increase in total cholesterol, LDL, VLDL, triglycerides and HDL.

At the end of the study (48^{th} day), the hydroalcoholic extracts of root of *Crateva magna* (100 mg/kg;p.o & 300 mg/kg;p.o) and Glibenclamide (10mg/kg;p.o) treated diabetic groups showed statistically significant decrease in total cholesterol, triglycerides, LDL, VLDL levels and increase in HDL level. So the hydroalcoholic extracts of *Crateva magna* showed the hypolipidemic activity.

Eugenia jambolana

7.1.3. ORAL GLUCOSE TOLERANCE TEST (OGTT)

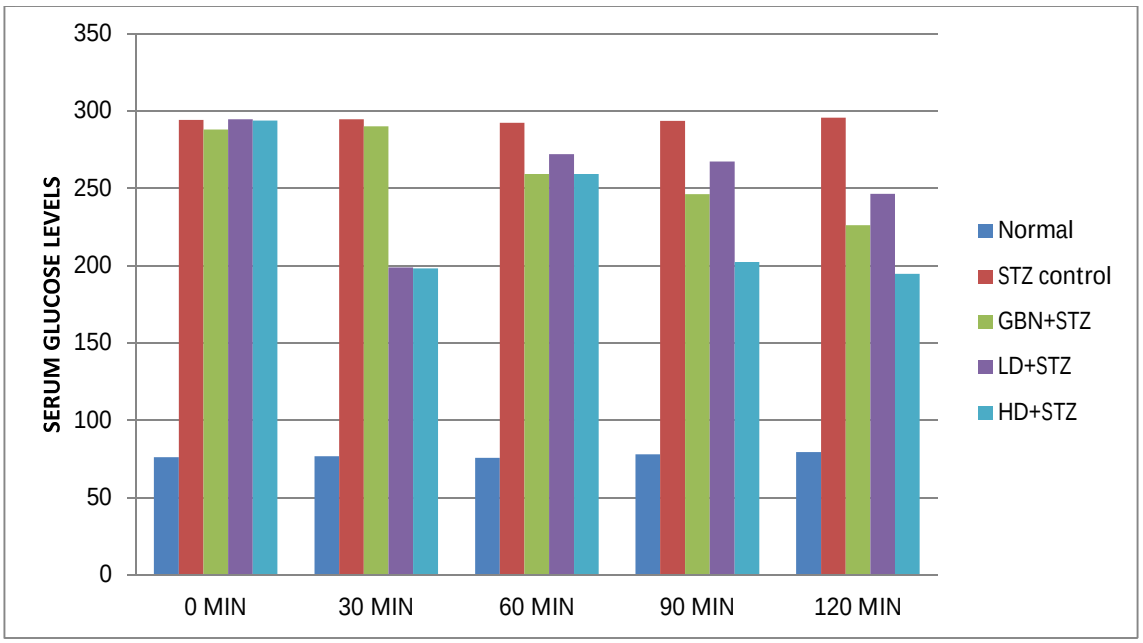
The effect of different doses of Hydroalcoholic extract of *Eugenia jambolana* seed on oral glucose tolerance test in normal rats.

Table no: 14. Results of effects of HAEEJS on OGTT

Group	Treatment	SERUM GLUCOSE (mg/ml)				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
1	Normal control	76.23± 1.68	76.86±1.52	75.90± 1.32	78.32± 1.45	79.70± 1.27
2	STZ treated diabetic control	294.5± 6.51	294.89±6.45	292.43± 6.53	293.91± 6.72	295.9± 6.87
3	Glibenclamide(10mg/kg;p.o) + glucose (2 gm/kg;p.o)	288.3± 8.21	290.43±6.56	259.50± 5.92	246.32± 4.54	226.31± 3.60
4	HAEEJS (100 mg/kg;p.o) + glucose (2 gm/kg;p.o)	294.86± 5.75	199.12±5.67	272.30±3.52**	267.45± 3.79	246.53± 3.81
5	HAEEJS (300 mg/kg;p.o) + glucose (2 gm/kg;p.o)	294.13± 8.65	198.28±6.26	259.5± 5.53	202.67±3.82**	194.81± 3.51

The values were estimated as Mean+S.E.M (n=6). Extract treated, Glibenclamide treated and glucose treated were compared with the vehicle control and STZ treated. **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.

Graph no: 8. Diagramatic representation of results of the effects of HAEEJS on OGTT



GBN-Glibenclamide(10mg/kg;p.o), GLU-Glucose(2gm/kg;p.o), HD-High dose(300mg/kg;p.o) of extract, LD-low dose(100mg/kg;p.o), STZ-Streptozotocin

Results

In Glucose Tolerance Test, the Glucose levels were estimated at 0,30,60,90 and 120 Mins respectively. The blood sugar level of extract treated groups were found compared to be diabetic control and the effects were dose dependant. Group II & IV glucose lowering efficiency between 90-120 Mins & were comparable to diabetic standard shown in table.

The experiment showed that OGTT measures the ability to use glucose, the bodys main source of energy, used to diagnose pre & post diabetes, glucose lowering effects were found after oral administration of extracts in rats may be due to the presence of hypoglycaemic flavanoids, terpenes or saponins that also requiring further investigation.

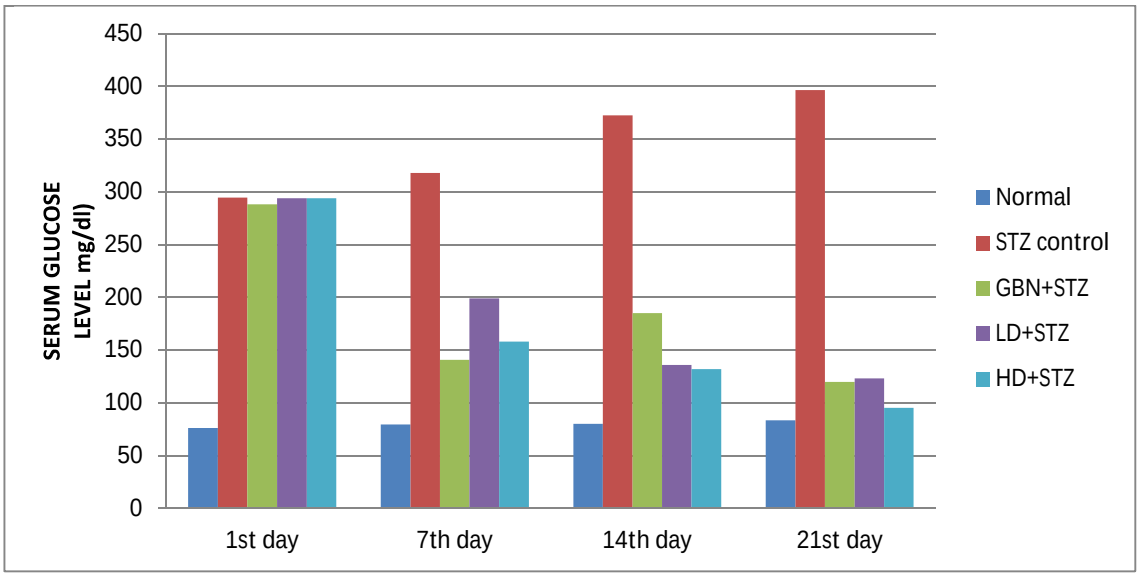
7.1.4. ESTIMATION OF BIOCHEMICAL PARAMETERS

7.1.4.a. Estimation of blood glucose

Table no: 15. Results of the effects of HAEEJS on serum glucose levels

Group	Treatment	Serum glucose levels (mg/dl)			
		0^{st} day	**7^{th} day**	**14^{th} day**	**21^{st} day**
1	Normal control	76.1± 5.52	79.6±1.96	80.3±1.64	83.45±1.72
2	STZ treated diabetic control	294.47± 6.33***	318±8.36***	372.21±10.299***	396.40±8.65***
3	Glibenclamide(10 mg/kg;p.o) + STZ	288.31± 12.04	140.8±5.71**	185.1±7.74**	120.21±7.22**
4	HAEEJS (100 mg/kg;p.o) + STZ	294±5.75**	199.63± 3.63***	136.52±11.30**	123.20±9.63**
5	HAEEJS (300 mg/kg;p.o) + STZ	294± 8.65	158.11±5.54***	132.03±3.35**	95.25±7.84***

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph No 9 Diagramatic representation of results of the effects of the HAEEJS on Serum glucose levels

STZ- Streptozotocin control,

GBN- Glibenclamide(10mg/kg;p.o)

LD-low dose(100mg/kg;p.o) of extract

HD-high dose(300mg/kg;p.o) of extract.

RESULTS

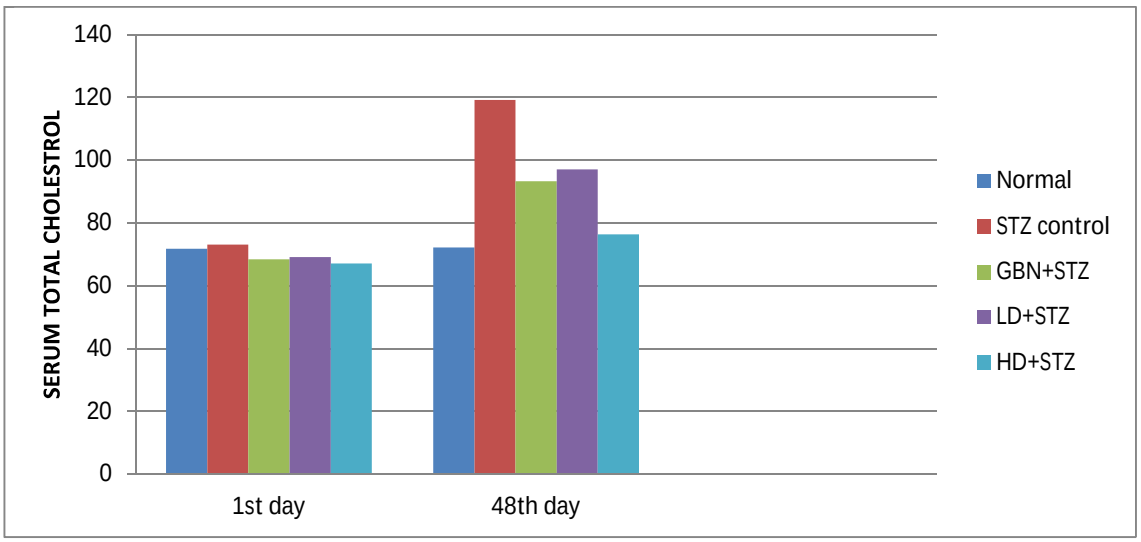
The blood glucose levels were measured on 0, 7^{th}, 14^{th}, 21^{st} days were showed in Table no. 7. When compared with normal control, STZ treated diabetic control group on 0, 7^{th}, 14^{th} and 21^{st} days showed significant ($p<0.001$) values. When compared to STZ treated diabetic control group, Glibenclamide(10mg/kg;p.o)+STZ treated diabetic group showed significant decrease in blood glucose levels on 7^{th}, 14^{th}, 21^{st} day($p<0.05$). When compared to STZ treated diabetic control group HAEEJS (300mg/kg;p.o) showed significant decrease in blood glucose levels($p<0.05$).

7.1.4.b ESTIMATION OF TOTAL CHOLESTEROL

Table no: 16. Results of the effects of HAEEJS on serum total cholesterol levels

Group	Treatment	Serum total cholesterol (mg/dl)	
		1st day	**48th day**
1	Normal control	71.68±5.12	71.24±7.10
2	STZ treated control	73.31±6.35	118.20±16.11
3	Glibenclamide (10 mg/kg;p.o) + STZ	68.54±3.10	94.33±10.1
4	HAEEJS (100 mg/kg;p.o) + STZ	70.21±6.12	96.08±7.32
5	HAEEJS (300 mg/kg;p.o) + STZ	67.19±4.12	75.4±4.59*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 10. Diagramatic representation of the results of the effects of the HAEEJS on total cholesterol levels

STZ- Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

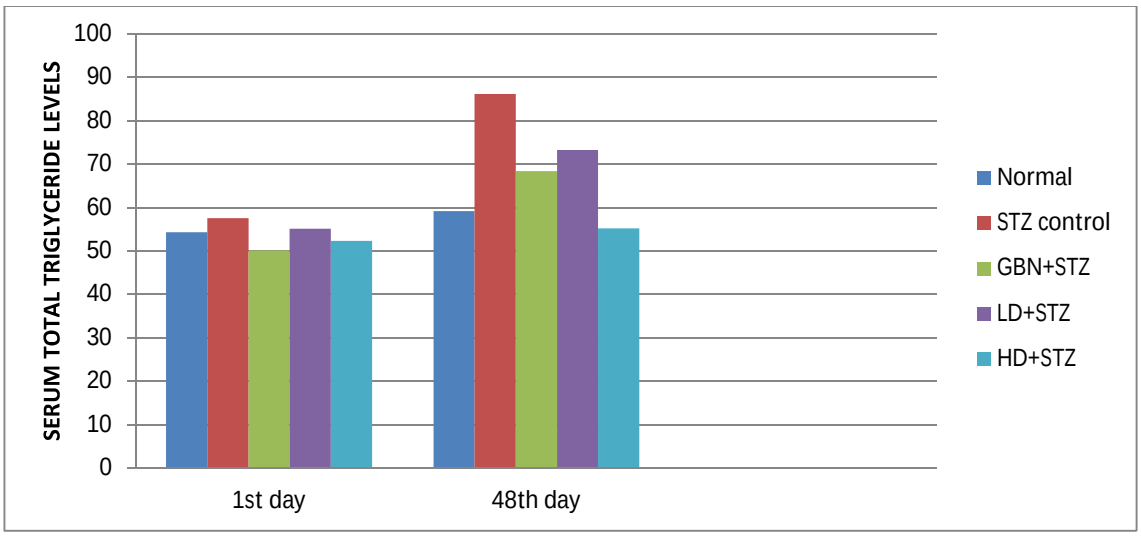
When compared with the STZ treated diabetic control group, Glibenclamide+STZ treated group showed significant decrease in total cholesterol levels($p<0.05$). When compared with STZ treated diabetic group control group and Glibenclamide+STZ treated group HAEEJS (300mg/kg;p.o) group showed significant decrease in total cholesterol levels($p<0.05$).

7.1.4.c. ESTIMATION OF TRIGLYCERIDES

Table no: 17. Results of the effects of HAEEJS on serum triglyceride levels

Group	Treatment	Serum triglycerides (mg/dl)	
		1st day	**48th day**
1	Normal control	54.72±3.61	58.10±4.13
2	STZ treated control	57.57±3.13	85.51±2.29
3	Glibenclamide(10mg/kg;p.o) + STZ	50.29±2.91	67.32±3.91
4	HAEEJS (100 mg/kg;p.o) + STZ	55.02±7.2	72.91±8.01
5	HAEEJS (300 mg/kg;p.o) + STZ	52.34±5.22	54.21±3.11*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 11. Diagramatic representation of the results of the effects of the HAEEJS on TG levels

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

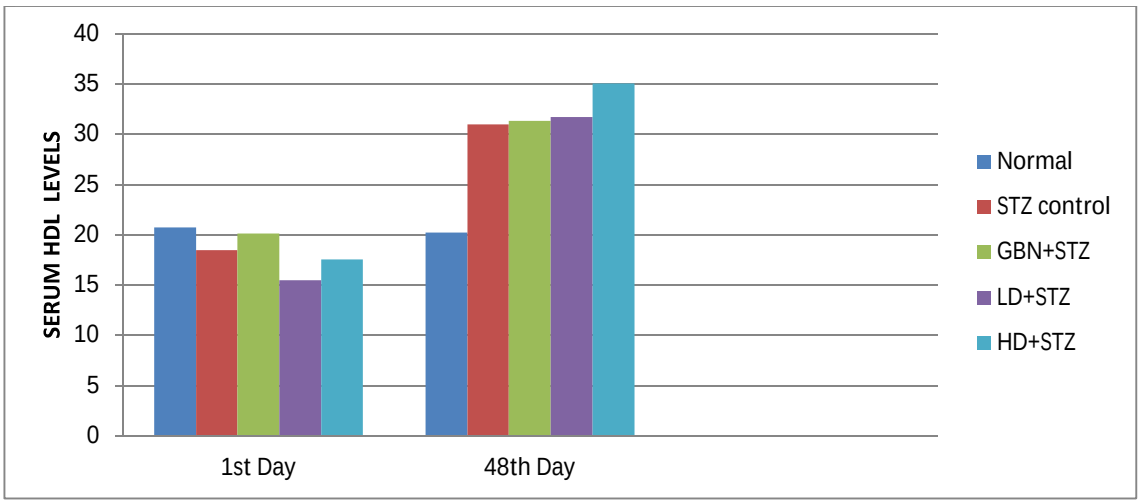
When compared with the STZ treated diabetic control group, Glibenclamide+STZ treated group showed significant decrease in Serum triglycerides level($p<0.05$). When compared with STZ treated diabetic control group and Glibenclamide+STZ treated group HAEEJS (300mg/kg;p.o) showed significant decrease in Serum triglycerides level($p<0.05$).

7.1.4.d. ESTIMATION OF HDL

Table no: 18. Results of the effects of HAEEJS on serum HDL levels

Group	Treatment	Serum HDL (mg/dl)	
		1st day	**48th day**
1	Normal control	20.82±3.46	20.13±2.58
2	STZ treated control	18.59±4.26	30.21±3.41
3	Glibenclamide(10mg/kg;p.o) + STZ	20.17±3.70	30.34±2.69
4	HAEEJS (100 mg/kg;p.o) + STZ	15.52±4.71	30.71±2.90
5	HAEEJS (300 mg/kg;p.o) + STZ	17.61±3.82	34.26±3.06*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 12. Diagramatic representation of results of HAEEJS on HDL

STZ-Steptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

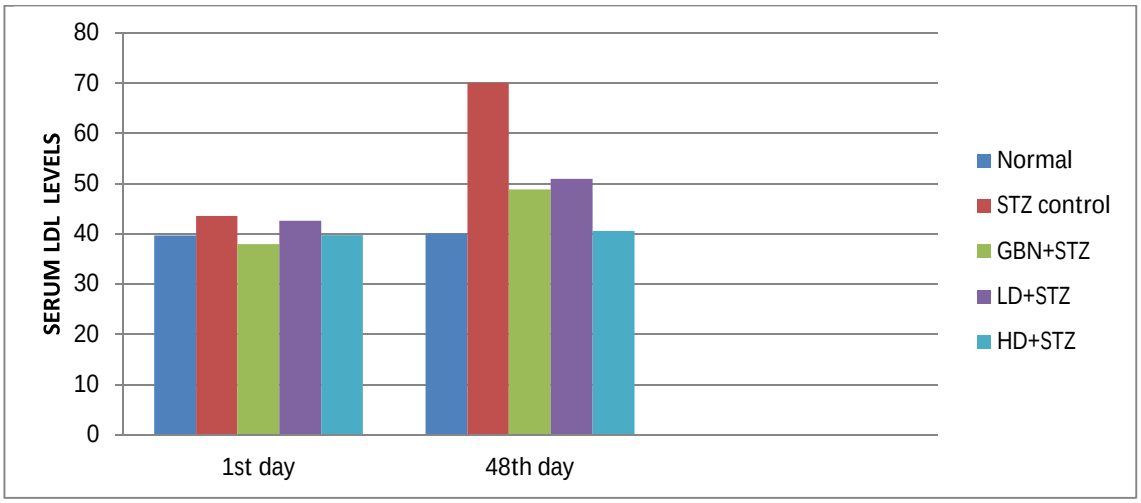
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAEEJS (300mg/kg;p.o) showed significant increase in HDL levels($p<0.05$).

7.1.4.e. ESTIMATION OF LDL

Table no: 19. Results of the effects of HAEEJS on serum LDL levels

Group	Treatment	Serum LDL (mg/dl)	
		1st day	**48th day**
1	Normal control	39.81±5.14	40.87±3.82
2	STZ treated control	43.67±4.69	69.26±12.0
3	Glibenclamide(10mg/kg;p.o) + STZ	38.01±5.14	47.97±8.32
4	HAEEJS (100 mg/kg;p.o) + STZ	42.75±2.58	50.03±3.51
5	HAEEJS (300 mg/kg;p.o) + STZ	39.72±2.65	40.71±0.03*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 13. Diagramatic representation of results of effects of HAEEJS **on LDL levels**

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

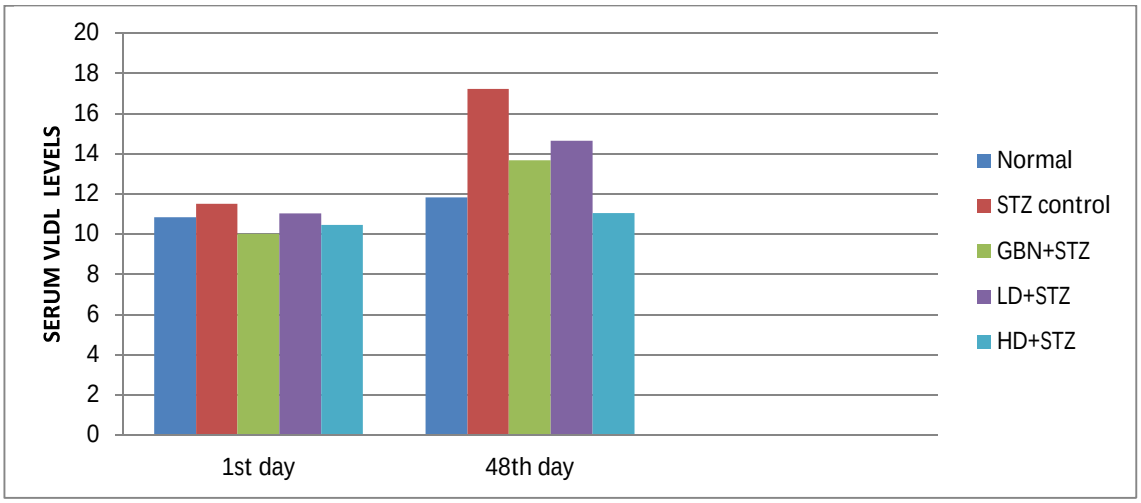
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAEEJS (300mg/kg;p.o) showed significant decrease in LDL levels.

7.1.4.f. ESTIMATION OF VLDL

Table no: 20. Results of the effects of HAEEJS on serum VLDL levels

Group	Treatment	Serum VLDL (mg/dl)	
		1st day	**48th day**
1	Normal control	10.81±0.73	11.62±0.87
2	STZ treated control	11.53±0.61	16.72±0.53
3	Glibenclamide (10mg/kg;p.o) + STZ	10.06±0.51	13.98±0.56
4	HAEEJS (100 mg/kg;p.o) + STZ	11.12±1.41	13.96±1.7
5	HAEEJS (300 mg/kg;p.o) + STZ	10.45±1.03	11.10±0.69*

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the vehicle control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant



Graph no: 14. Diagramatic representation of results of effects of HAEEJS on VLDL levels

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-low dose(100mg/kg;p.o) of extract, HD-high dose(300mg/kg;p.o) of extract.

RESULTS

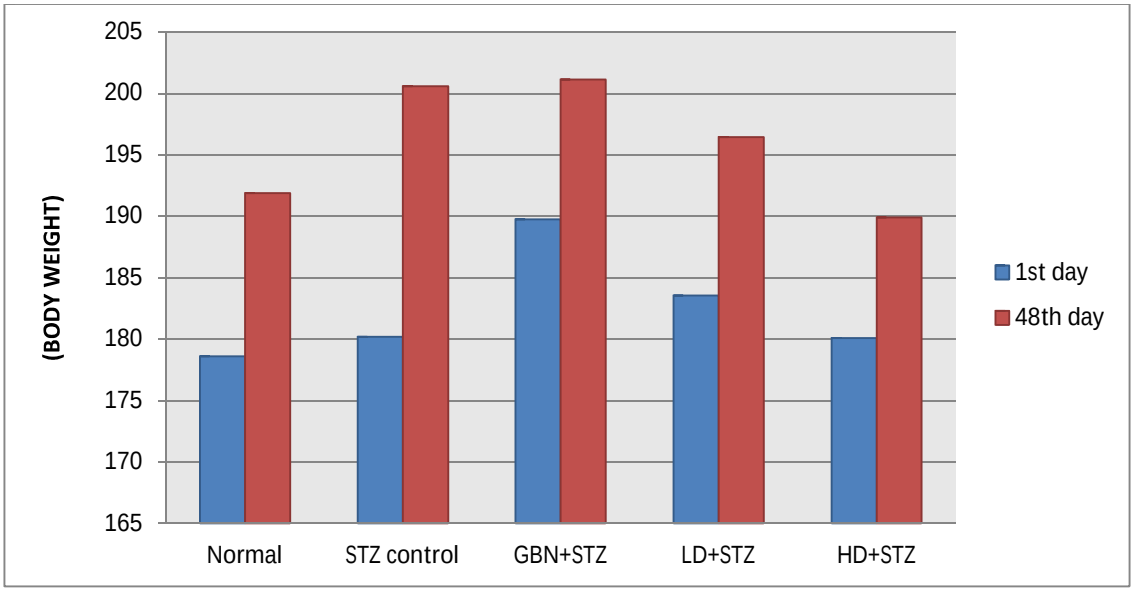
When compared to STZ treated diabetic control group and Glibenclamide+STZ treated group, HAEEJS (300mg/kg;p.o) showed significant decrease in VLDL levels($p<0.05$).

7.1.4.g. ESTIMATION OF BODY WEIGHT

Table no: 21. Results of the effects of HAEEJS on body weight

Group	Treatment	Body weight (gm)		
		0^{th} day	48^{th} day	Gain
1	Normal control	178.61 ±5.6	190.8±5.2	12.19±4.52
2	STZ treated control	180.18 ±3.31	201.67±4.14	21.49±5.01
3	Glibenclamide (60 µg/kg;p.o) + STZ	189.55 ±5.11	200.10±5.01	10.55±4.99
4	HAEEJS (100 mg/kg;p.o) + STZ	183.65 ±4.21	195.43±3.91	11.78±2.91
5	HAEEJS (300 mg/kg;p.o) + STZ	180.41 ±3.91	188.7*±7.07	8.29*±4.60

The values were expressed as Mean ± S.E.M. (n=6) by using ANOVA followed by Dunnet test. STZ treated control (Diabetic control) was compared with the normal control, extract treated and Glibenclamide treated. *** P<0.001-Statistically highly significant; **P<0.01-Statistically very significant; *P<0.05-Statistically significant; ns - Statistically not significant.



Graph no: 15. Diagramatic representation of results of effects of HAEEJS on body weight before and after treatment of standard and HAEEJS

STZ-Streptozotocin, GBN-Glibenclamide(10mg/kg;p.o),

LD-lower dose(100mg/kg;p.o) of extract, HD-higher dose(300mg/kg;p.o) of extract.

RESULTS

When compared with STZ treated diabetic control group, Glibenclamide+STZ group and HAECMR (300mg/kg;p.o) showed significant difference in the body weight($p<0.05$).

In addition to the above test we have calculated % fall in blood glucose level for 3 samples namely 1. Crateva magna, 2. Eugenia jambolana, 3. 1:1 mixture of these two and consequently ED 50 values for these 3 samples were obtained graphically (Refer below Table 22,23 and 24 graph no 16)

Table No 22 Crateva magna root (HAECMR)

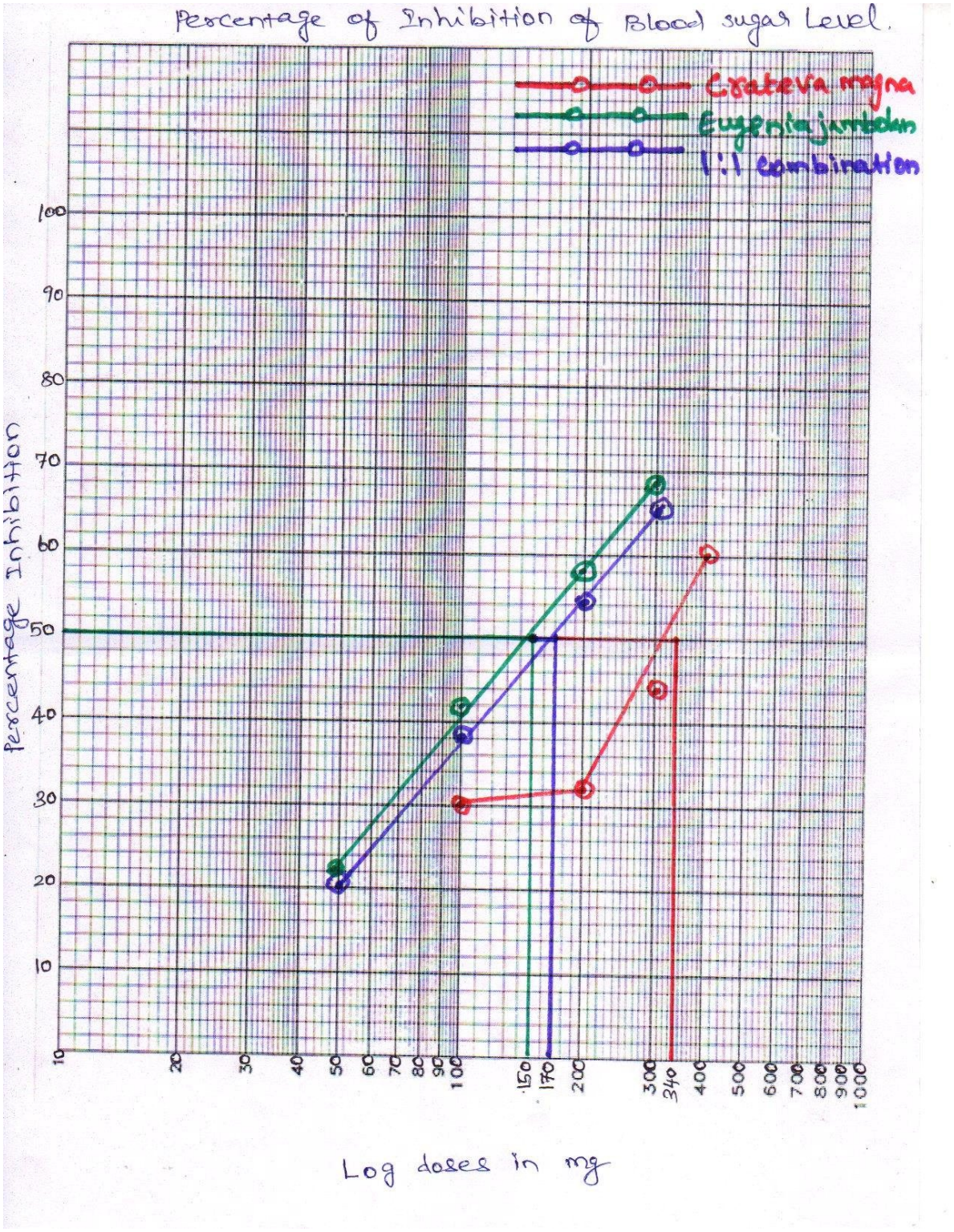
Doses in mg/kg,p.o	**0 Day**	**7th Day**	**14th Day**	**21st Day**	**% age fall after 21 days**
100	298.0±12.31**	248.63±7.33***	220.52±11.30**	185.20±9.63	30%
200	310.44±6.25	268.0±7.62**	212.0±6.85	175.40±5.92	32%
300	298.88±3.52**	235.11±5.50***	210.03±3.35**	185.71±7.84***	44%
400	298.12±5.98	210.45±6.21	188.74±4.35	119.22±6.25	60%

Table No 23 Eugenia jambolana seed (HAEEJS)

Doses in mg/kg,p.o	**0 Day**	**7th Day**	**14th Day**	**21st Day**	**% age fall after 21 days**
50	294.0±3.18	252.58±3.67	241.80±4.21	229.32±4.13	22%
100	294.0±5.12	199.0±3.63***	185.38±2.15***	170.60±4.45	42%
200	294.0±6.18	182.30±4.54	152.88±4.32	123.48±5.42	58%
300	294.0±6.78	158.0±3.84***	136.30±2.74***	95.25±3.75	68%

Table No 24 Crateva magna and Eugenia jambolana 1:1 combination (HAECOM)

Doses in mg/kg,p.o	**0 Day**	**7th Day**	**14th Day**	**21st Day**	**% age fall after 21 days**
50	295.15±5.12	280.15±3.62	257.23±4.18	236.15±5.24	20%
100	294.25±4.82	280.32±5.62	249.45±3.14	182.45±4.27	38%
200	296.75±3.98	274.34±4.67	221.27±5.51	136.50±5.02	54%
300	295.65±5.08	271.67±3.82	196.78±4.64	103.48±3.14	65%


Percentage of Inhibition of Blood sugar Level.
Crateva magna
Eugenia jambolana
1:1 combination
Percentage Inhibition
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100
150
170
200
300
340
400
500
600
700
800
900
1000
Log doses in mg

Graph No 16

Dose Response Curve

Graph No 16 shows the DRC of Crateva magna, Eugenia jambolana and 1:1 mixture of these two each at 4 dose level.

DRC of Crateva magna is shifted to the right hand side as compared to other 2 graphs. The DRC of Eugenia jambolana shifted to the left hand side which indicates Eugenia jambolana is most potent sample for its anti diabetic effect as compared to other two samples. The DRC of the mixture of these two seems to be comparatively more potent than Crateva magna alone as its graph has been shifted to the left hand side away from the Crateva magna graph and it lies very close to Eugenia graph.

8. SUMMARY AND CONCLUSION

The Hydroalcoholic extract of root of *Crateva magna* in a dose of 300mg/kg;p.o showed statistically significant antidiabetic and hypolipidemic activity.

The Hydroalcoholic extract of seed of *Eugenia jambolana* in a dose of 300mg/kg;p.o showed statistically significant antidiabetic and hypolipidemic activity.

The extract showed significant activity against Streptozotocin induced Diabetes in rats when compared with that of standard drug Glibenclamide, In percentage inhibition graph ED 50 dose of Crateva Magana which causes 50 % inhibition of the blood glucose level is found to 340 mg p.o and that of Eugenia jambolana 150 mg p.o and that of 1:1 mixture of these two is found to 170 mg.

Although the mixture does not seem to be more potent than Eugenia alone definitely this combination found to superior than Crateva magna alone.

We understand as per the literature survey Crateva magna reduces the blood sugar level by inhibiting by enzyme alpha glucosidase therefore Eugenia jambolana potentiates the antidiabetic effect of Crateva magna.

The extract showed significant activity against Streptozotocin induced Diabetes in rats when compared with that of standard drug Glibenclamide.

In conclusion, the results of the present study reveals the Antidiabetic and hypolipidemic activity against diabetes in rats.

Further investigation is underway to determine the exact phytoconstituents in the extract that are responsible for its Antidiabetic and hypolipidemic activity.

We recommend who so ever wants to continue this work to try upon other antidiabetic herbal drugs and its mixture with Crateva magna and in this way to introduce into the market newer and safer poly herbal anti diabetic formulation. Then only these findings will act as a boon for the humanity suffering from diabetics.

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